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PRELIMINARY STUDIES ON OOGENESIS IN A CYST-FORMING NEMATODE, *HETERODERA AVENAE* (NEMATODA: HETERODERIDAE) [1])

BY

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According to BAKER (1945) the oat cyst nematode, *Heterodera avenae* Filipjev (= *H. major*), which attacks some cereal crops, is rather widely distributed in southwestern Ontario. The nematode causes considerable damage to oats in this area. Males of this species are numerous. The following is a report on oogenesis in *H. avenae*, a subject which has not been previously investigated.

MATERIALS AND METHODS

An ample supply of gravid white females was available from mass cultures of the oat cyst nematode on the roots of Cartier oats grown in the greenhouse at Ottawa. The live females were prepared for study by the squash technique as described by MULVEY (1955). Ferric hydroxide was also used with propionic orcein in staining the chromosomes.

OOGONIAL DEVELOPMENT

Oogonial development followed closely that described by MULVEY (1955) for a species of *Heterodera* on hairy vetch(*Vicia villosa* Huds.). No prochromosomes were observed.

OOCYTES

Upon completion of the growth period the chromatin is organized into definite chromosomes. Duplication of each chromosome follows and then the homologous chromosomes pair. During first metaphase of meiosis the chromosome pairs arrange themselves at the equatorial pla-

1) Contribution No. 3798, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

ne (Figs. 1, 2). There are nine pairs of chromosomes, the same number being observed by RILEY & CHAPMAN (1957) in the golden nematode, *H. rostochiensis* Woll. and by MULVEY (1957) in the sugar-beet nematode, *H. schachtii* Schm. During early metaphase the spindle and its nuclear elements lie at the center of the egg. Eventually the chromosomes separate and the spindle, which is normally parallel to the long axis of the egg, rotates through an angle of 90° and comes to rest with one of its poles against the cell membrane. The first polar body is then formed (Figs. 3, 4, 9). During this time the sperm (Fig. 7) remains near the periphery of the egg but its chromatin mass has begun to resolve into individual chromosomes. Eventually a second polar body is formed (Figs. 5, 6, 10).

FERTILIZATION

The small, tailless, and numerous sperm are contained in a rounded spermatheca (Fig. 8). The sperm enters the egg during early metaphase of the first maturation division and remains apart from the egg nucleus until the second polar body is formed. Fusion of the egg and the sperm nuclei was not observed.

ZUSAMMENFASSUNG

Vorläufige Studien über die Oogenese in einem cystenbildenden Nematoden, Heterodera avenae *(Nematoda: Heteroderidae)*

Bei den Reifeteilungen in den Weibchen des Hafercystennematoden, *Heterodera avenae* (= *major*), bildeten sich zwei polare Körper. Die haploide Zahl der Chromosomen betrug neun. Die zahlreichen kleinen, schwanzlosen Spermien sind in einer runden Spermatheca im Innern des Ovars aufbewahrt. Nur ein Sperma wurde in jeder Oocyte, die wir untersuchten, gefunden.

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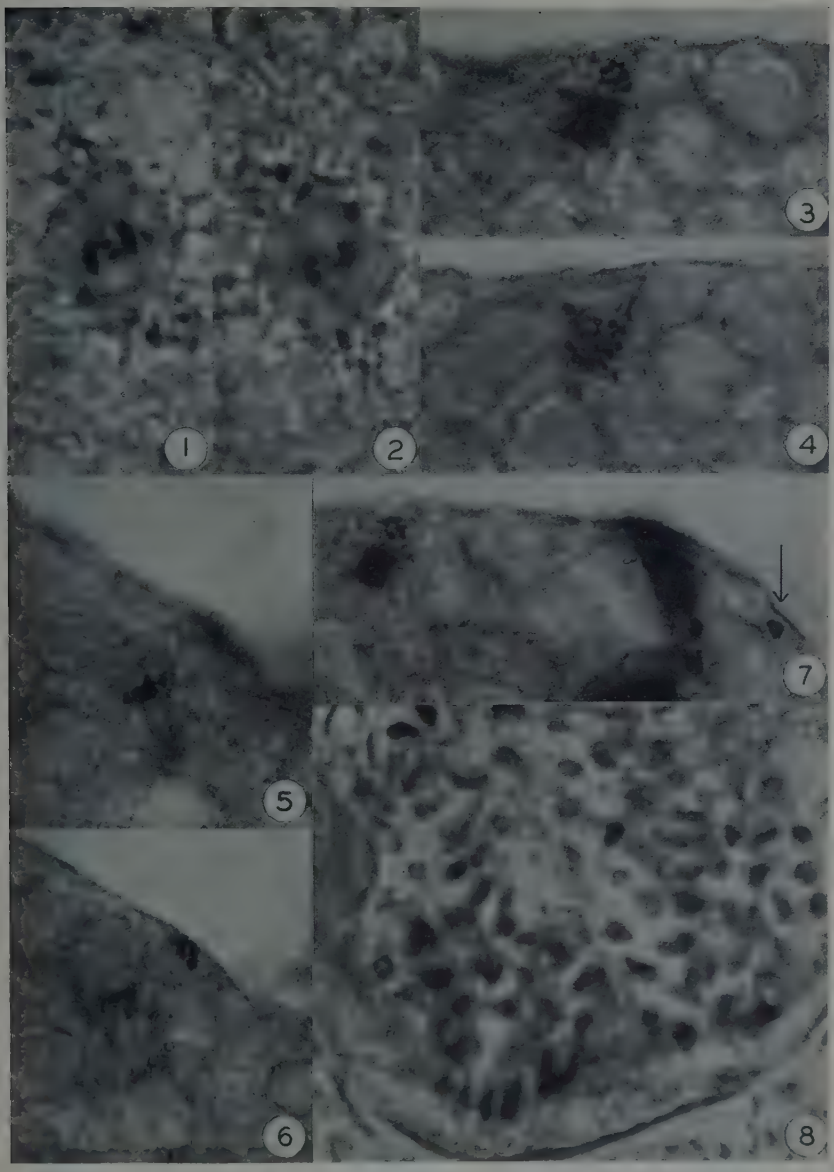
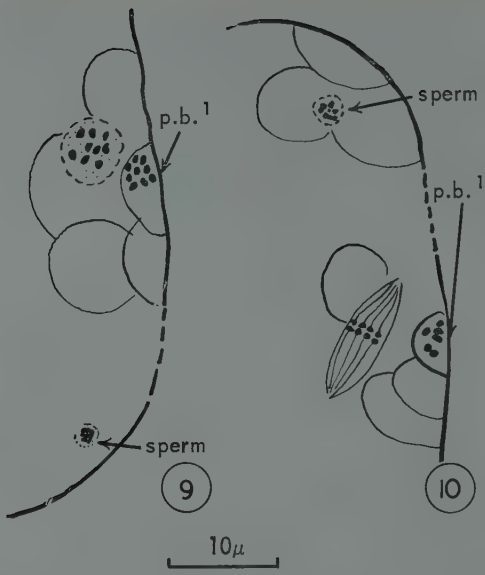
R. H. MULVEY: *Oogenesis in* Heterodera avenae.



Figs. 1-8
Legends overleaf



Figs. 9-10. Camera lucida drawings showing finer details of Figs. 3-4 and 5-6 respectively.

Fig. 1-8. Photomicrographs of maturation divisions in *Heterodera avenae*. X 1500. 1, 2 Chromosomes (tetrads) at metaphase plate, first maturation division. Side view showing spindle and thin cell membrane. Photographed at two levels. 3, 4 Telophase stage showing formation of first polar body (toward outside of oocyte) and dyads of oocyte (inside of oocyte). There are nine chromosomes in each group. 5, 6 Second maturation division showing first polar body nearly outside and the spindle with dyads at metaphase plate, which will form second polar body. 7 Sperm shown near periphery of oocyte (indicated by arrow). The first polar body and telophase group of the oocyte are in the upper-left corner. 8 Spermatheca, showing sperm.

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UNTERSUCHUNGEN ÜBER *PARATYLENCHUS AMBLYCEPHALUS* N. SP. (NEMATODA, CRICONEMATIDAE) [1])

VON

I. REUVER

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Bei den von SWART-FÜCHTBAUER (1954) an Apfel beobachteten parasitären Nematoden handelt es sich nach meinen Untersuchungen um eine neue, bisher nicht bekannte *Paratylenchus*-Art, für die ich nach ihrer Kopfform den Namen *amblycephalus* vorschlage.

Paratylenchus amblycephalus besitzt die typischen Merkmale der von MICOLETZKY (1922), THORNE (1949) und GOODEY (1951) beschriebenen Gattung *Paratylenchus*: schlanke bewegliche Form; fein geringelte Kutikula; langer schlanker Stachel, der bei Männchen rückgebildet ist oder ganz fehlt; Weibchen mit einem Ovar; Männchen ohne Bursa.

Weibchen: n = 25; Länge 378 (301-442) μ, Breite 16 (13-21) μ, a = 24,1 (20,4-27,9), β = 3,9 (3,6-4,6), γ = 14,7 (11,4-16,7), V = 83 (77-87) %, Stachellänge 32 (30-34) μ.
Zahl der Ringe hinter der Vulva: 48-57.

Kopf stumpf zulaufend, oben flach abgeplattet (Abb. 1 a u. c). Die Ausstosstelle des Stachels ist schwach erhöht. Der lange schlanke Mundstachel endet in einer scharfen Spitze. Stachelknöpfe gut ausgebildet. Leitschiene des Stachels ein verdickter Ring. Der Ösophagus ist typisch. Die Dorsaldrüse mündet 7 μ hinter den Stachelknöpfen in das Ösophaguslumen ein. Darmlumen wegen Ablagerung von Fettkugeln in den Eingeweiden nur selten zu sehen. Diese Reservestoffe häufig in Abständen zu Gruppen vereinigt, welche die Tiere abwechselnd dunkel und hell erscheinen lassen. Anus auf ca. $^2/_3$ des Abstandes Vulva-Schwanzspitze, oft nur schwach sichtbar. Exkretionsporus ventral in der Höhe des Basalbulbus. Kurz davor oder an derselben Stelle ist das Hemizonid erkennbar.

1) Auszug aus der Dissertation „Untersuchungen über *Paratylenchus amblycephalus* n. sp. (Nematoda, Criconematidae) und seine Beziehung zur Baumschulmüdigkeit", Bonn 1958.

Das Ovarium ist ausgestreckt und zieht sich unter Umständen bis in die Höhe des mittleren Ösophagusbulbus hin. Ein postvulvarer Uterussack fehlt. Junge Weibchen besitzen ein sehr grosses, fast viereckiges, dicht mit Spermien gefülltes Receptaculum seminis, während es bei älteren Weibchen, die bereits viele Eier abgelegt haben, kleiner und rund ist (Abb. 1 b). Der Uterus kann nur ein Ei zur gleichen Zeit aufnehmen. Vagina kräftig ausgebildet, Vulva ein transversaler Schlitz mit zwei vorstehenden Lippen. Reife Weibchen, bei denen infolge des stark ausgebildeten Ovars die Körperbreite vor der Vulva beträchtlich zugenommen hat, zeigen eine stärkere obere Vulvalippe. Die laterale Vulvamembran ist dann von den Vulvalippen zum Teil überdeckt, während sie bei jungen Weibchen sehr gut sichtbar ist.

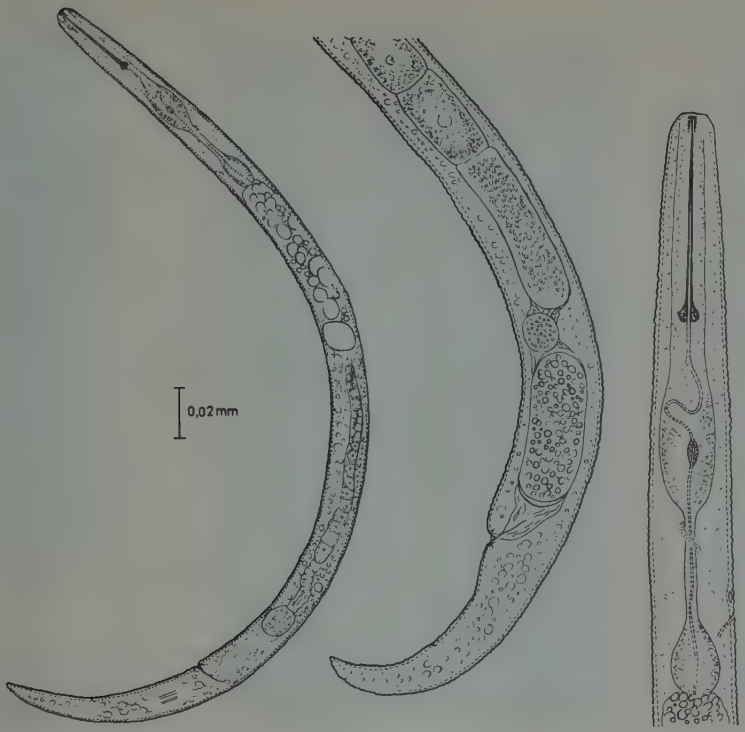
Querringelung der Kutikula gleichmässig, am Kopf- und Schwanzende schwächer ausgebildet. Jeder Ringel 1-2 μ breit. Auf beiden Seiten der Tiere, abgesehen von der Stachelregion, sind 4 Seitenlinien gut zu verfolgen. Sie nehmen ca. $^1/_3$ der Körperbreite ein. Vor der Vulva, an der grössten Breite des Körpers, weichen die Linien allmählich auseinander, um unmittelbar anschliessend in engerem Abstand bis zur Schwanzspitze zu verlaufen.

Die jungen Weibchen sind schlank und ähneln in Körperform und Bewegung den meisten anderen bekannten *Paratylenchus*-Arten, die reifen sind infolge des ausgebildeten Ovars in ihren Bewegungen schwerfälliger. Schwanz plump, in der Ruhelage ventralwärts gekrümmt. Spitze verschieden geformt (Abb. 1 d). Deiridien und Phasmidien nicht beobachtet.

Männchen: n = 27; Länge 384 (335-435) μ, Breite 12,7 (12,1-13,5) μ, α = 30,1 (26,1-35,4), β = 4,3 (3,7-4,7), γ = 13,7 (11,7-16,5). Spicula 21 μ, Gubernaculum 4 μ, Schwanzringe 25-27.

Männchen annähernd ebenso gross wie, aber schlanker als die Weibchen; sie zeigen ebenfalls die für *Paratylenchus* typischen Bewegungen. Kopf wie bei den Weibchen stumpf und flach. Mundstachel und Ösophagus stark degeneriert, Bulben nur ganz schwach entwickelt (Abb. 1 e). Exkretionsporus und das auffällige Hemizonid meistens gut zu erkennen.

Einfache bis zu 80 μ lange Gonade in der Regel dicht mit Spermien gefüllt. Die ventral gebogenen Spicula und das Gubernaculum einfach gebaut (Abb. 1 e). Phasmidien nicht beobachtet. Schwanzspitzen wie beim weiblichen Geschlecht verschieden geformt. Männchen kommen verhältnismässig häufig vor.



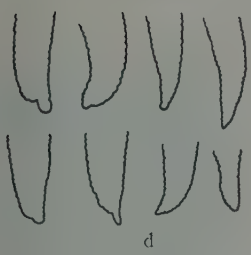


Abb. 1. *Paratylenchus amblycephalus*. a. Weibchen 350 ×, b. Schwanzende 750 ×, c. Vorderende 800 ×, d. verschieden ausgebildete Schwanzspitzen 700 ×, e. Männchen, Vorder- und Schwanzende 800 ×.

Larven (L_1-L_3): n = 27; Länge 151-327 μ, Breite 8,1-13,5 μ, α = 15,5-26,8 β = 2,2-4,3, γ = 10,0-16,6. Mundstachel 16-24 μ.

Kopfende der Larven wie bei den Adulten. Bei älteren Larvenstadien wird der Wert für α grösser, u.a. dadurch, dass die Körperbreite abnimmt. Junge Larvenstadien haben einen sehr langen, ältere einen kürzeren Ösophagus im Vergleich zur Körperlänge, was sich in einer Zunahme von β äussert. Bei kleinen wie bei grösseren Larven konnte der Mundstachel beobachtet werden. Ösophagusregion, ebenso wie die übrigen Eingeweideteile, meistens schlecht zu erkennen.

Larven gelegentlich schon im Ei fertig entwickelt. Ob es sich dabei um das erste oder zweite Larvenstadium handelte, wie OOSTENBRINK (1953) es für *Paratylenchus goodeyi* angibt, konnte nicht festgestellt werden.

Neben stacheltragenden Larven wurden oft solche mit einem unscheinbaren kurzen, schwach geknöpften Mundstachel gefunden, die nach Kopfform und Bewegungsart ebenfalls zur Gattung *Paratylenchus* gehören (Abb. 2). Da ausser *P. amblycephalus* bei den Untersuchungen keine andere *Paratylenchus*-Species auftrat, musste es sich um Larven der hier beschriebenen Art handeln.

Larven (L_4): n = 10; Länge 347 (316-407) μ, Breite 15,2 (13,5-16,2) μ, α = 22,9 (20,1-25,1), β = 4,3 (3,9-4,9), γ = 14,1 (13,0-15,4), Mundstachel 16 μ.

Diese Larven wirken plumper im Gegensatz zu denjenigen mit normal ausgebildetem Mundstachel.

Eier: (43,2-48,6) μ x (18,9-21,6) μ.

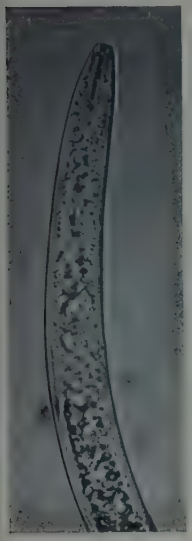
In den im Uterus befindlichen Eiern keine ausgebildeten Larven beobachtet. Sie zeigten eine nicht differenzierte Struktur, wie auch die meisten abgelegten Eier.

Typ-Exemplare: Holotyp: Weibchen l_4; Allotyp: Männchen l_5; Paratypen: l_{6-13}, alle in der Sammlung Plantenziektenkundige Dienst, Wageningen.

Wirtspflanzen: An Wurzeln von *Malus* EM IV, daneben *Malus* EM IX, *Sorbus aucuparia* und *Cydonia* A.

Fundort: Frauweiler bei Bedburg/Erft, Reg. Bez. Köln, Deutschland.

Artabgrenzung: *Paratylenchus amblycephalus* ist aus folgenden Gründen mit keiner der bisher beschriebenen *Paratylenchus*-Arten als identisch anzusehen: *P. bukowinensis* Micoletzky, 1922, *P. besoekianus* Balley & Reydon, 1931, *P. curvitata* van der Linde, 1938, *P. elachistus* Steiner, 1949, *P. minutus* Linford, Oliveira & Ishii 1949 und *P. dianthus*



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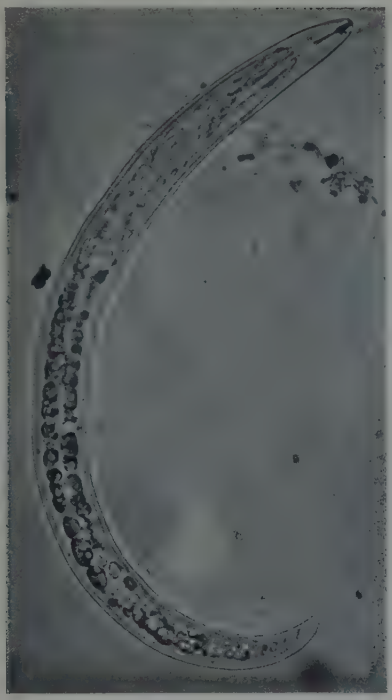

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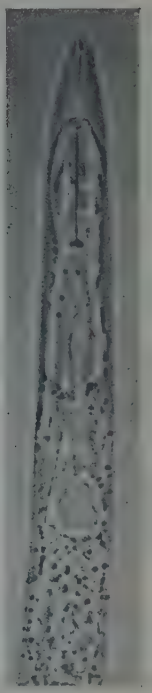

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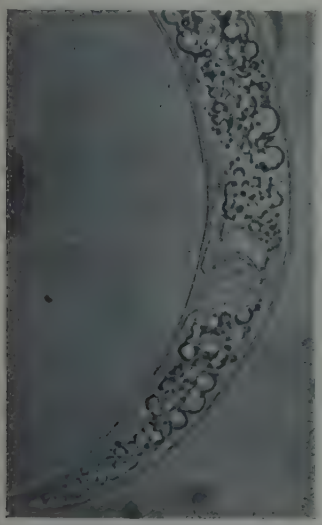
Abb. 2. *P. amblycephalus,* letztes Larvenstadium 550 ×. — Abb. 3. Weibchen in der alten Larvenhaut, 400 ×. — Abb. 4. Weibchen in der alten Larvenhaut, Vorderende, 600 ×.



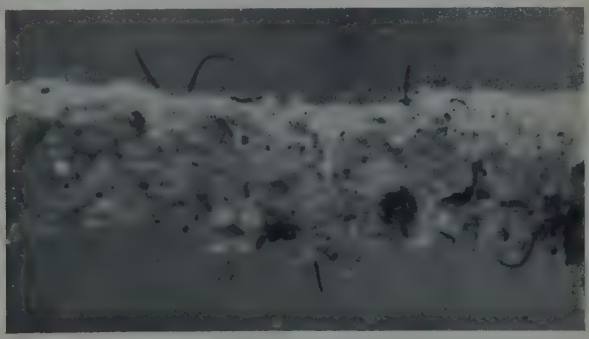


Abb. 6. Von *P. amblycephalus* befallene Faserwurzel mit Eiablage, 50 ×.

← Abb. 5. Umwandlung der Larve zum Weibchen, Bildung der Vagina und Vulva, 600 ×.

Jenkins & Taylor, 1956 haben einen kürzeren, *P. anceps* Cobb, 1923 und *P. goodeyi* Oostenbrink, 1953 einen längeren Stachel. Für *P. anceps* und *P. nanus* Cobb, 1923 sowie *P. projectus* Jenkins, 1956 wurden keine Männchen nachgewiesen. *P. goodeyi* zeichnet sich durch die stacheltragenden Männchen sowie durch andere Kopfform und Körpergrösse aus. Die Angaben über *P. macrophallus* (De Man, 1880) Goodey, 1934 gehen sehr auseinander. Die Männchen besitzen einen Mundstachel; den Weibchen fehlt die Vulvamembran neben anderen Unterscheidungsmerkmalen. *P. hamatus* Thorne & Allen, 1950, dessen Stachellänge der von *P. amblycephalus* nahekommt, unterscheidet sich durch die stacheltragenden Männchen und den abgerundeten Kopf. Die Weibchen von *P. projectus* besitzen kein Receptaculum, aber eine abgesetzte, mit 3 Ringen versehene Lippenregion.

Hauptcharakteristika von *P. amblycephalus* sind der stumpfe flache Kopf, die Stachellänge von 32 μ, das häufige Auftreten von Männchen, die die gleiche Kopfform wie die Weibchen haben, aber stachellos sind.

UNTERSUCHUNGEN ZUR BIOLOGIE

a. Untersuchungen über die Larvenentwicklung.

Nach Angabe von Maupas (1899, zit. in Filipjev & Schuurmans Stekhoven, 1941) haben die Nematoden 5 Entwicklungsstadien, die von 4 Häutungen unterbrochen werden.

Wie erwähnt, wurden gelegentlich im Ei von *P. amblycephalus* ausgebildete Larven beobachtet. Die kleinsten an den Wurzeln bzw. im Boden gefundenen waren ca. 150 μ, die grössten 300 μ lang. Nimmt man den zwischen den Häutungen nicht an Länge zunehmenden Stachel als Kriterium für die Anzahl der Larvenstadien, so lassen sich 3 Stadien unterscheiden, deren Mundstachel 16-18 μ, bzw. 20-21 μ, bzw. 23-24 μ misst. Häutungsstadien konnten verschiedentlich beobachtet werden. Zunächst liess sich die untere Stachelpartie mit den Knöpfen nicht mehr erkennen. Nur der vordere mit der alten Kutikula abgestossene Teil des Stachels blieb sichtbar. Öfters konnte das neue Stadium in der alten Kutikula beobachtet werden. Letztere hebt sich zunächst am Kopf- und Schwanzende ab und zwar erst dann, wenn sich Mundstachel und Organe neu differenziert hatten (Abb. 3 u. 4).

Ferner wurden Häutungsstadien gefunden, bei denen sich das neue Larvenstadium schon von der Kutikula abgehoben hatte, aber nur den vorderen Teil des Mundstachels erkennen liess. Hierbei musste es sich um die Häutung zu dem Larvenstadium mit dem schwachen kurzen Mundstachel handeln, das, vermutlich wegen das unzureichend ausge

bildeten Stachels, nie an den Wurzeln saugend beobachtet wurde. Dass dieses das letzte Larvenstadium ist, kann aus verschiedenen Gründen angenommen werden. Einmal schliessen die innerhalb des letzten Larvenstadiums vor sich gehenden Veränderungen zum Geschlechtstier möglicherweise eine Nahrungsaufnahme aus und erklären das Fehlen eines normalen Mundstachels. Ferner wurden verschiedentlich Häutungen zum Geschlechtstier beobachtet, bei denen die sich häutenden Larven im Aussehen den beschriebenen Larven entsprachen. Schliesslich sind diese grösser als die stacheltragenden Formen und unterscheiden sich von den adulten Tieren in ihrer Länge nur geringfügig.

Die sich häutenden Larven fielen durch Bewegungslosigkeit und gekrümmte Lage auf. Bei manchen konnte man die Bildung von Vulva und Vagina verfolgen, die sich nach Auflösung des Gewebes als unausgekleidete Hohlräume erkennen liessen (Abb. 5). Gelegentlich liess sich die Abhebung der alten Larvenhaut in der ganzen Länge des Tieres verfolgen.

Im allgemeinen bilden sich Vulva bzw. Spicula auf der Ventralseite des alten Larvenstadiums aus. In zwei Fällen, bei einem Weibchen und einem Männchen, konnte jedoch beobachtet werden, dass die Vulva bzw. die Spicula auf der nach aussen gekrümmten Seite lagen. Bei dem Männchen war daher alte bzw. neue Analöffnung, bei dem Weibchen alter und neuer Exkretionsporus sowohl auf der Ventral- als auch auf der Dorsalseite der Larve zu sehen.

b. Untersuchungen zur Entwicklungsdauer.

Für die Entwicklung wandernder Nematoden haben Untersuchungen von Linford & Oliveira (1940) an *Rotylenchus reniformis* Anhaltspunkte gegeben. Sie beobachteten die *Rotylenchus*-Entwicklung von der Eiablage bis zum reifen Tier im Wasser bzw. an Pflanzenwurzeln in kleinen Glaskästen, die eine direkte Kontrolle der Nematoden erlaubten.

Ich versuchte, nachdem direkte Beobachtung der Entwicklung in kleinen Glaskästen sowie in Wasser sich als ungeeignet erwiesen hatte, durch Wurzeluntersuchungen an Apfelsämlingen Einblick in die Entwicklung von *P. amblycephalus* zu erhalten. Junge Apfelsämlinge im 2-3-Blattstadium wurden in Blumentöpfe mit *Paratylenchus* verseuchtem Boden gepflanzt und im Gewächshaus bei ca. 20° C aufgestellt. Von den 15 bis 20 Töpfen jeder Versuchsreihe wurden wöchentlich die Wurzeln von 3-4 Sämlingen zuerst im Wasser und anschliessend nach Anfärbung mit Lactophenol-Baumwollblau untersucht. Die Ergebnisse sind in Tabelle I zusammengestellt.

Tabelle I

Beobachtung der Entwicklungsdauer von P. amblycephalus *an Apfelsämlingen, Bonitierung des Wurzelbefalls (Weibchen-Eier-Larven)*

Kontrolle nach Tagen	Wiederholungen 1	2	3	4
7	o	Weibchen	Weibchen	Weibchen
14	Weibchen	Weibchen	Weibchen	Weibchen
	+ Eiablage		+ Eiablage!	+ Eiablage
21	Weibchen	Weibchen	Weibchen	Weibchen
	+ Eiablage	+ Eiablage!	+ Eiablage	+ Eiablage
28		Weibchen	Weibchen	
		+ Eiablage	+ Eiablage	
		+ Larven	+ Larven	
35				Weibchen
				+ Eiablage
				+ Larven

o = kein Befall
! = Larven in Eiern kurz vor dem Schlupf

Da der Boden nicht immer gleich stark verseucht war, ergaben sich Unterschiede im Befall der Wurzeln. Mit Hilfe der Baumwollblau-Färbung liessen sich nach 14 Tagen bei 3 der Wiederholungen mehrfach Eiablagen, oft in unmittelbarer Nähe saugender Weibchen, nachweisen; demnach werden die Eier während des Saugens abgelegt (Abb. 6). Ihre Anzahl schwankt zwischen 3 und 12. Einzelne Eier könnten sich beim Färben aus den Gelegen gelöst haben, so dass ihre Zahl auch noch etwas höher sein kann. Meist liess sich keine differenzierte Struktur beobachten; nur in Versuch 3 waren einzelne ausgebildete Larven an der typischen Schlingenbildung in der Eihülle, die auf baldiges Schlüpfen hindeutet, zu erkennen. Am 28. Tage wurden in Versuch 2 und 3, am 35. Tag in Versuch 4 an verschiedenen Stellen junge, an den Wurzeln saugende Larven zu mehreren an einer Stelle gefunden, zuweilen in unmittelbarer Nähe eines alten Weibchens. In welchem Zeitraum *P. amblycephalus* seine Geschlechtsreife erreicht, konnte nicht ermittelt werden.

c. Beobachtungen bei Infektionsversuchen.

Durch Infektionsversuche mit *P. amblycephalus* strebte ich eine Anreicherung der im Freiland in geringen Mengen vorkommenden Art an und wollte gleichzeitig Material für weitere Untersuchungen gewinnen, um unabhängiger vom Vorkommen im Freiland zu sein. Ferner sollten Vermehrungsfähigkeit sowie von den Nematoden hervorgerufene Symptome an den Wurzeln beobachtet werden. Junge Apfel-

sämlinge der Sorte „Bittenfelder Sämling" wurden in Sand angezogen und im 2-3-Blattstadium in sterilisierten müden Boden in Blumentöpfe von 6 cm ø umgesetzt. In den Boden wurde eine Suspension einer gemischten Älchenpopulation mittels Pipette in Wurzelnähe eingebracht. Zur Herstellung der Suspensionen wurden die aus mehreren Erdproben isolierten Älchengemische vereinigt und das überschüssige Wasser nach Absinken der Nematoden abgesaugt; die Suspensionen enthielten dann 200-500 *P. amblycephalus* und in geringen Mengen *Pratylenchus penetrans, Tylenchorhynchus macrurus, Rotylenchus robustus, Tylenchus* sp. sowie saprophytische Nematoden. Jeweils 2-3 Apfelsämlinge wurden als Kontrolle mit der gleichen Menge nematodenfreien abgesaugten Wassers behandelt. Um zu prüfen, ob sich aus Eiern eine *Paratylenchus*-Population heranziehen lässt, wurden Apfelsämlingen Wurzeln mit Eiablagen hinzugefügt, von denen die saugenden Nematoden nach 1-2 tägigem Stehenlassen in Wasser abgefallen waren. Alle Versuchspflanzen standen im Gewächshaus bei Temperaturen von 15° bis 20° C. Zeitpunkt der Infektionen und Zahl der infizierten Sämlinge richtete sich nach der Menge der gerade zur Verfügung stehenden *Paratylenchus*-Suspension. Durchschnittlich konnten 3-6 Parallel-Infektionen vorgenommen werden.

Die Pflanzen wurden erst nach mehreren Monaten untersucht, da sich diese Zeitspanne als notwendig für die Vermehrung der Nematoden erwies. Die Auswertung von Wurzel- und Bodenbefall erfolgte getrennt. Bei sehr kräftig entwickeltem Wurzelballen wurden an 2-3 Stellen Wurzelteile, bei spärlichem Wurzelwachstum das ganze Wurzelsystem untersucht.

Befallene Pflanzen unterschieden sich in Trieb- und Wurzellänge nicht von unbefallenen. Die Ausbildung des Wurzelsystems war bei den Kontrollpflanzen gleichmässiger, während die befallenen Pflanzen in der oberen Hälfte vermehrte Seitenwurzelbildung zeigten. Die Wurzelspitzen endeten häufig mit einer Verdickung, sahen aber im allgemeinen frisch aus. Nekrosen von punktartigen braunen Verfärbungen bis zur Grösse von einigen mm traten sowohl an Kontroll- als auch an befallenen Pflanzen — bei letzteren häufiger — auf. Sie werden oft auf Nematodenbefall zurückgeführt (Ark & Thomas 1936, Chitwood 1949, Oostenbrink 1954). Ich fand sie jedoch häufig an Wurzeln, an denen keine Nematoden saugten; andererseits zeigten zweifellos schon länger befallene Stellen keine Veränderung des Gewebes.

Auffällig war der überwiegende Befall an den oberen Wurzelpartien. Hier wurden nicht nur an jungen und frisch gebildeten Saugwurzeln

sondern auch an schon verholzten Wurzelteilen, — sogar an der kräftigen Hauptwurzel, oft dicht unter der Erdoberfläche — grosse Mengen von Nematoden, vorwiegend Larvenstadien, beobachtet. Auch der in der Erde befindliche Teil des Hypokotyls war von jungen Larven befallen, nicht jedoch von älteren Stadien und ausgewachsenen Tieren.

An den unteren Partien des Wurzelballens fanden sich nur bei sehr starkem Befall Nematoden. Junge Wurzeln dieser Region mit vielen Wurzelhaaren wurden von *P. amblycephalus* offensichtlich gemieden.

Der Befall an den Wurzeln war also nicht gleichmässig über das Wurzelsystem verteilt. Manche Wurzeln waren sehr dicht besetzt — auf 2 mm Länge zählte ich über 50 Nematoden — andere vollkommen frei. Vielfach sammelten sich Larven in unmittelbarer Nähe eines ausgewachsenen Weibchens. Häufig hatten mehrere Weibchen bzw. Larven ihren Kopf gleichzeitig an ein und derselben Wurzelstelle eingebohrt. Bevorzugt sassen die Nematoden an mit Sand behafteten Wurzelpartien. An den äussersten Wurzelspitzen beobachtete ich nur selten saugende Nematoden. Männchen wurden an den Wurzeln nie, jedoch im Boden oft in erheblichen Mengen gefunden.

Bei den Auszählungen wurden Weibchen, Männchen und Larven getrennt bewertet. Die Ergebnisse sind in Tabelle II zusammengestellt.

Tabelle II

Wurzelbefall und Anzahl P. amblycephalus *120 ccm Boden nach künstlicher Infektion mit Suspensionsgemischen an Apfelsämlingen*

Infektion m. *Parat.*	unters. n. Mon.	Wurzelbefall *	Weibchen	Männchen	Larven	gesamt
ca. 400	14	5	ca. 300	ca. 550	ca. 650	1500
„ „	14	5	„ 235	637	214	1086
„ „	14	5	„ 600	„ 1000	„ 1000	2600
„ „	14	5	„ 250	„ 600	„ 600	1450
„ „	13	5	„ 340	„ 340	„ 500	1180
„ „	13	2	40	63	83	186
„ 500	13	5	„ 250	„ 290	„ 670	1210
„ 200	13	1	26	121	38	185
„ 500	13	1	34	360	60	454
„ „	13	1	22	62	8	92
„ „	12	4	112	394	104	510
„ 400	10	2	112	203	114	419
„ „	10	0	5	101	4	109

*) 0 = kein Befall
1 = nur vereinzelt saugende Nematoden
2 = schwacher Befall
3 = mittelstarker Befall
4 = starker Befall
5 = sehr starker Befall

Wie aus Tabelle II hervorgeht, können die Männchen den Weibchen zahlenmässig um das 2-3 fache, im Extremfall sogar um das 20-fache überlegen sein, Verhältnisse, die im Freiland nie festgestellt wurden. Da nach Filipjev & Schuurmans Stekhoven (1941) unter ungünstigen Umweltbedingungen mehr Männchen auftreten können als unter normalen, ist ihr starkes Überwiegen vielleicht hieraus zu erklären. Möglicherweise sind auch durch Verschlechterung der Lebensbedingungen viele Weibchen zugrunde gegangen. Andererseits ist mit fehlender Nahrungsmöglichkeit für die Weibchen nicht zu rechnen, da auch bei schwer befallenen Pflanzen immer noch leicht oder nicht befallene Wurzeln vorhanden waren.

Umgerechnet auf das Volumen des Blumentopfes (100 ccm) vermehrte sich in einigen Töpfen die Gesamtpopulation ausserordentlich stark, im Extremfall auf das 32-fache der Ausgangspopulation. Die Anzahl der Larven überstieg fast immer die der Weibchen, während sie teils niedriger, teils höher als die der Männchen war.

Eigenartig war das Auftreten vieler Larven mit schwachem Mundstachel. Sie befanden sich oft in grossen Mengen in den ausgewaschenen Suspensionen, auch wenn nur wenige Weibchen, Männchen und stacheltragende Larven vorhanden waren. Ihre Zahl überstieg bei manchen Untersuchungen das 10-20 fache der stacheltragenden Larven. Für ihre Zugehörigkeit zu *P. amblycephalus* spricht, dass sie auch nach Infektion mit isolierten Tieren gefunden wurden. Ein mit Männchen und den fraglichen Larven infizierter Apfelsämling war nach 3 Monaten an verschiedenen Wurzeln von stacheltragenden Larven und einigen Weibchen befallen.

Von Infektionen, die mit einer abgezählten Menge isolierter *P. amblycephalus* angesetzt waren, gingen nur wenige an. Nach solchen mit weniger als 100 Tieren trat kein Befall ein. Die positiv ausgefallenen Infektionen zeigten wiederum zum Teil eine Vermehrung auf das Vielfache der Ausgangspopulation.

Apfelsämlingen in sterilisiertem Boden, denen mit Eiablagen behaftete Wurzeln zugefügt waren, zeigten in einem Fall nach drei Monaten an den Wurzeln saugende Larven, in einem anderen nach 6 Monaten Weibchen und Larven. Bei der Bodenuntersuchung wurden im letzten Fall alle Entwicklungsstadien von *P. amblycephalus* gefunden, im ersten nur vereinzelte Tiere. In den meisten in gleicher Weise durchgeführten Versuchen war weder Wurzel- noch Bodenbefall festzustellen.

UNTERSUCHUNGEN ZUR ÖKOLOGIE

Temperaturversuche mit Apfelsämlingen im 2-3-Blattstadium in *Paratylenchus*-verseuchtem Boden sollten zeigen, in welchem Zeitraum die Nematoden bei 4°, 10°, 16° und 21°C die Wurzeln vom Boden aus zu befallen vermögen. Der erste Befall war bei 21°- und 16°-Pflanzen durchweg nach 2-4 Tagen festzustellen, während 4° und 10° ihn gegenüber den erstgenannten Temperaturen bis zu 8 Tagen verzögerten. Nach weiteren Beobachtungen scheint sich die anfänglich geringere Befallsstärke bei niederen Temperaturen allmählich der höherer Temperaturen anzugleichen. Die Vermehrung wird offenbar durch höhere Temperaturen (16°, 21°) beschleunigt.

Wie die Temperatur die Aktivität von *P. amblycephalus* beeinflusst, wurde bei 4°, 7°, 12°, 16°, 21°, 26° und 32° C in Leitungswasser geprüft. Dabei zeigte sich, dass 50 % der Tiere bei 32° schon nach 4 Tagen, bei 7° erst nach 46 Tagen inaktiv waren; bei 4° und 12° waren es 33 bzw. 32 Tage, bei 16° 27 Tage, bei 21° und 26° 20 bzw. 11 Tage. Bei 7° und 12° blieben einzelne Nematoden mehr als 100 Tage am Leben. Temperaturen von 36°, 38°, 42° und 46° wurden nur zwischen 4 und ½ Stunde lang ertragen.

In Laborversuchen wurde geprüft, wie Temperaturen zwischen 0° und —8° bei einer Einwirkungsdauer von 7 bis 28 Tagen und einer Bodenfeuchtigkeit zwischen 5 und 20 % (des lufttrockenen Bodens) auf die Lebensfähigkeit von *P. amblycephalus* wirken. Mit zunehmender „Kältesumme" (Temperatur × Einwirkungszeit) nahm die Zahl der Überlebenden um so stärker ab, je höher der Wassergehalt des Bodens war. Andererseits geht bei gleichbleibend geringer Kältesumme (—4° × 7 Tage) die Population bei niedrigem Wassergehalt nicht, bei hohem jedoch stark zurück. Doch wurde sie selbst bei hoher Kältesumme (—8° × 23 Tage) und 20 % Bodenfeuchtigkeit nicht völlig vernichtet. Kältesumme und Wassergehalt wirken demnach gemeinsam als auch für sich auf die Lebensfähigkeit von *P. amblycephalus* ein.

Versuche mit zunehmend ausgetrocknetem Boden ergaben eine allmähliche, wenn auch in den einzelnen Wiederholungen unterschiedlich starke Abnahme der Lebensfähigkeit von *P. amblycephalus*. Erst unter 6 % Bodenfeuchtigkeit ging die Population erheblich zurück; bei 2 % waren alle Tiere tot. Weibchen scheinen weniger empfindlich als Männchen und Larven zu sein.

Die Untersuchungen wurden mit Unterstützung des Ministeriums für Ernährung, Landwirtschaft und Forsten in Düsseldorf durchgeführt, dem für die grosszügige Bewilligung von Mitteln ergebenst gedankt sei.

Herrn Dr. M. Oostenbrink danke ich herzlich für die Unterstützung bei der Art-Abgrenzung von *Paratylenchus amblycephalus*, besonders aber für sein in allen Fragen gewährtes Entgegenkommen.

SUMMARY

Studies on Paratylenchus amblycephalus *n. sp.* (*Nematoda, Criconematidae*)

A description of *Paratylenchus amblycephalus*, n. sp. on apple roots is given. *P. amblycephalus* is characterised by a flat, blunt head; female stylet 32 μ; males numerous, without stylets. Four free larval stages were observed, the fourth with a reduced stylet. Eggs were found 7 days after the roots were attacked and larvae emerged after 14 days. Populations increased greatly on infested apple seedlings; males were often predominant. Symptoms caused by attacks of *P. amblycephalus* on the roots of apple seedlings are described. Soil temperatures of 21° and 16°C favoured on attack more than 10° or 4°C. Temperatures below 0°C and high soil moisture content reduced the longevity of the nematodes, which were found to be relatively resistant to desiccation of the soil.

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NOUVEAUX CRICONEMATIDAE DE LA ZONE INTERTROPICALE (NEMATODA : TYLENCHIDA)

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Au cours de l'examen de nombreux échantillons de sol et de racines de végétaux provenant de l'Ouest Africain et de Madagascar nous avons fréquemment rencontré des espèces appartenant aux genres *Hemicycliophora* De Man 1921 [1]), *Criconemoides* Taylor 1936 et, plus rarement, *Criconema* Hofmänner & Menzel 1914 et *Hemicriconemoides* Chitwood & Birchfield 1957.

Dans tous les cas l'espèce observée n'était pas le seul Tylenchida présent dans l'échantillon et ne représentait qu'un taux généralement assez faible du peuplement nématologique parasitaire. Les différentes espèces observées ne semblent donc pas être des parasites à grand développement mais faire toujours partie d'un complexe parasitaire où leur part numérique n'est pas prépondérante. La plupart des individus ont été rencontrés uniquement dans le sol; cependant de nombreuses femelles de *Criconemoides onoense* n. sp. ont été observées piquées dans les racines d'ananas (*Ananas sativus* Schult.) par leur long stylet; les individus sont solitaires et leur point de pénétration le long des jeunes racines semble indifférent.

A la suite d'observations prolongées sur le vivant il semble bien qu'il faille abandonner la conception classique de Stauffer (1924) sur le mode de progression des *Criconemoides* et *Criconema*. D'après cet auteur les représentants de ces genres progresseraient par télescopage des anneaux les uns dans les autres vers l'avant, puis par extension en prenant appui sur la partie arrière du corps et de nouveau télescopage vers l'avant. Nous n'avons jamais observé de faits semblables; par contre tous les individus rencontrés étaient parfaitement capables de

1) Les espèces appartenant au genre *Hemicycliophora* ont déjà fait l'objet d'une publication: (Luc, M. (1958). Trois nouvelles espèces africaines du genre *Hemicycliophora* De Man 1921 (Nematoda, Criconematidae), *Nematologica*, 3, 15-23).

se replier en C ouvert, en cercle même et d'avoir, pour les grandes espèces surtout, des mouvements d'ondulation, moins prononcés que chez les *Tylenchidae* certes, mais parfaitement capables de les faire progresser sans recourir à un schéma particulier que rien ne parait justifier ni dans leur comportement ni dans leur anatomie.

Les différentes espèces rencontrées ont été les suivantes:

Criconemoides onoense n. sp., parasite sur ananas (*Ananas sativus* Schult.) dans toutes les plantations de Côte d'Ivoire. Egalement rencontré au voisinage de racines de bananier (*Musa paradisiaca* L. var. *sapientum*) à Kindia (Guinée).

Criconemoides citri Steiner 1949, au voisinage de racines de Bananier-Plantain (*Musa paradisiaca* L.) à Nossy-Bé (Madagascar); de racines de Canne à Sucre (*Saccharum officinarum* L.) à Ambilobé et à Namakia-Majunga (Madagascar); de Manioc (*Manihot utilissima* Pohl.) à Marovitsiki (Madagascar); de Cotonnier (*Gossypium hirsutum* L. var. Acala 4-42) à Miary-Tuléar (Madagascar).

Criconemoides ferniae n. sp. au voisinage de racines d'ananas à Ambodirana-Mangoro (Madagascar).

Criconemoides curvatum Raski 1952 au voisinage de racines de Riz (*Oryza sativa* L.) à Kankan (Guinée) et de Mil à Chandelle (*Pennisetum typhoideum* Rich.) à Adiopodoumé (Côte d'Ivoire).

Criconema limitaneum n. sp., au voisinage de racines de Quinquina (*Cinchona succirubra* Pav.) à Sérédou (Guinée).

Hemicriconemoides cocophilus (Loos 1949), Chitwood 1957, au voisinage de racines d'ananas, à Akoupé (Côte d'Ivoire).

Nous donnons ci-dessous la description des trois espèces nouvelles et quelques précisions sur la partie antérieure des *Criconemoides citri*.

Criconemoides onoense n. sp.

(Pl. I, E-G)

Dimensions: Femelles: L: 0,389-0,494 mm. a: 11,3-14,7, b: 4,1-5,4, c: 18,7-20,4. V: 91,4-94,2 %.

Description: Femelle: Habitus, sur animal fixé, droit ou très peu courbé. Nombre d'anneaux: 128-136; la plupart des animaux ne présentent aucune anastomose latérale entre les anneaux; quand il en existe, c'est une seule (exceptionnellement deux) et généralement sur la partie postvulvaire, l'anneau incomplet pouvant être ventral ou dorsal indifféremment. Tête arrondie; disque labial peu en relief; lobes labiaux petits mais nettement détachés du disque et sur le même plan que celui-ci. Deux premiers anneaux non retrorses, plus étroits que les

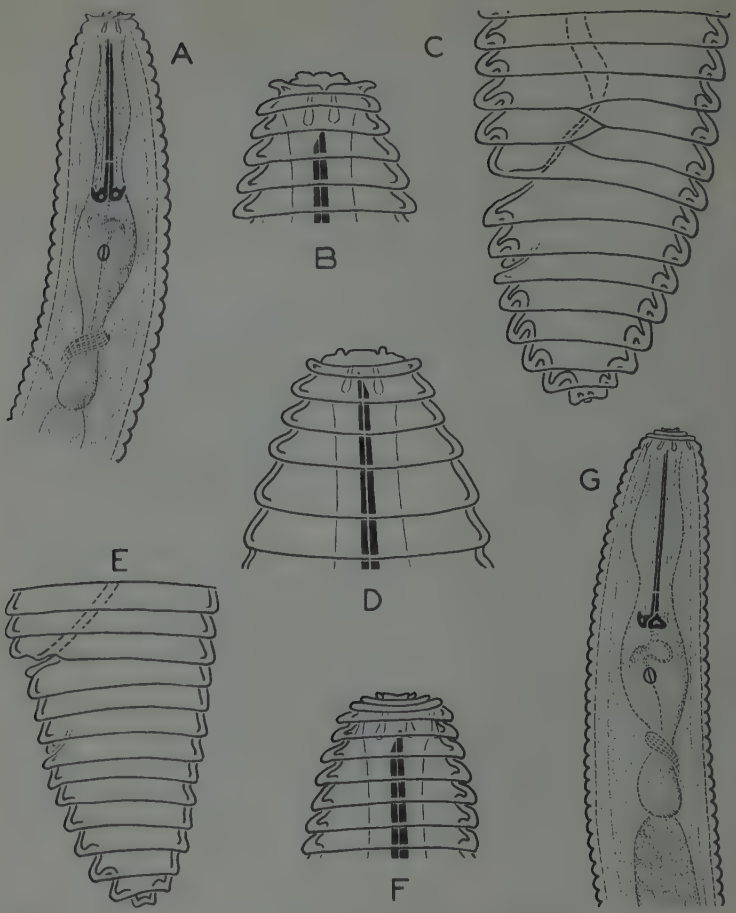

Planche 1. *Criconemoides ferniae* n. sp.: A: partie antérieure. B: détails des premiers anneaux. C: partie post-vulvaire.
Criconemoides citri Steiner 1949: D: détail des anneaux antérieurs.
Criconemoides onoense n. sp.: E: partie post-vulvaire. F: détail des premiers anneaux. G: partie antérieure.
(Gross.: A et G: × 550. B, C, D, E et F × 1.100)

anneaux du corps et légèrement en cuvette mais sans incisure les séparant des anneaux postérieurs; premier anneau non divisé, le bord antérieur tout au plus légèrement ondulé; sur quelques rares exemplaires le troisième anneau était également non retrorse mais d'un seul côté. Stylet

long de 40-45 μ; boutons basaux avec procès antérieurs relativement aigus. Pore excréteur situé sur le 31ème anneau. Vulve située à 8-11 anneaux de l'extrémité postérieure; lèvre antérieure bilobée. Portion post-vulvaire conique — allongée; extrémité obtuse avec anneau terminal de forme variable.

Mâle: inconnu.

Holotype: Lame 02. Laboratoire de Nématologie I.D.E.R.T. Abidjan, Côte d'Ivoire. Paratype déposé au laboratoire de Nématologie de l'I.P.O. à Wageningen (Pays-Bas).

Hôte type: racines de *Ananas sativus* Schult.

Lieu type: Plantation Salci, à Ono (Côte d'Ivoire).

Diagnose: *C. onoense* n. sp., d'après la clé de Raski (1952), est voisin de *C. curvatum* Raski 1952. Il s'en différencie par: l'habitus presque rectiligne, le nombre nettement plus grand des anneaux du corps et le premier anneau toujours continu.

Les individus de *C. onoense* n. sp. observés au voisinage des racines de bananier (*Musa paradisiaca* L. var. *sapientum*) à Kindia (Guinée) diffèrent légèrement de la description ci-dessus en plusieurs points: le nombre d'anneaux est légèrement plus faible (114-124); le stylet est un peu plus long (49-55 μ) et le nombre d'anastomoses plus grand (4 à 6). Cependant les autres dimensions, la structure de la partie antérieure, la forme de la partie postvulvaire étant identiques nous considérons ces deux formes comme appartenant à la même espèce.

Criconemoides ferniae n. sp.

(Pl. I, A-C)

Dimensions: Femelle: L: 0,372. a: 11,1, b: 3,5, c: 17,7. V: 91,8 %.

Description: Femelle: Habitus, sur animal fixé, courbé ventralement. Nombre d'anneaux: 93 ventralement et 95 dorsalement ceci par suite de deux anastomoses. Tête à apparence tronquée; disque et lobes labiaux sur le même plan, ces derniers peu détachés du disque. Premier anneau non retrorse mais très légèrement en coupe vers l'avant, séparé des autres anneaux par une incisure marquée et divisé en deux par deux fentes latérales. Stylet long de 45 μ s'étendant sur 15 anneaux. Pore excréteur situé sur le 27ème anneau. Oesophage s'étendant jusqu'au 30ème anneau. Vulve située à 8 anneaux de l'extrémité postérieure; lèvre antérieure simple. Anus situé à 5 anneaux de l'extrémité postérieure. Queue arrondie, quelque peu déviée dorsalement.

Mâle: inconnu.

Holotype: Lame 03. Laboratoire de Nématologie. I.D.E.R.T. Abidjan,

Côte d'Ivoire.
Lieu type : Sol, voisinage des racines de *Ananas sativus* Schult., Ambodirana (Madagascar).
Diagnose : *C. ferniae* n. sp. s'accorde d'après la clé de Raski (1952) avec *C. lobatum* Raski 1952; il s'en différencie essentiellement par les caractères de la partie antérieure :

— lobes labiaux beaucoup moins développés.

— premier anneau divisé en deux parties dorso-ventrales et non en quatre plaques labiales dont les dorsoventrales réduites.

— longueur du corps plus faible (0,372 : 0,402-0,480 mm).

Criconemoides curvatum Raski 1952

Les individus rencontrés ne diffèrent de la description originale que par leur stylet plus court : 43-47 μ contre 47-67 μ. Tous les autres caractères concordant nous les rangeons cependant dans cette espèce.

Criconemoides citri Steiner 1949

(Pl. I, D)

Les représentants de cette espèce qui semble particulièrement répandue à Madagascar, concordent parfaitement avec la description originale. Nous compléterons celle-ci par quelques précisions sur la partie antérieure : disque labial peu en relief, lobes labiaux dépassant ce dernier mais non nettement détachés; ensemble de ces différentes pièces bombé, saillant nettement du premier anneau qui est continu, non retrorse, plus étroit que les anneaux du corps mais ne présente pas d'incisure le séparant de ces derniers.

Criconema limitaneum n. sp.

(Pl. II)

Dimensions : Femelles : L : 0,207-0,228 mm. a : 5,8-7,6, b : 2,5-2,6, c : 16,3. V : 87,4-89,0 %.
Description : Femelles : Premier anneau non retrorse mais en forme de coupe profonde masquant tout le détail des pièces labiales externes. Deuxième anneau mince, ondulé à sa jonction avec le premier. Stylet long de 52-53 μ, caractéristique du genre, avec appendices peu en relief. Pore excréteur sur le 34ème anneau à 96 μ de l'avant. Hémizonide (?) : petite zone réfringente juste en avant du pore excréteur. Cuticule marquée par des anneaux retrorses, le nombre de ces anneaux différant sur le côté dorsal et ventral par suite d'anastomoses le plus souvent incomplètes débutant toujours sur le champ latéral qui n'est pas autrement marqué. Le nombre des anneaux est de 90-110 ventrale-

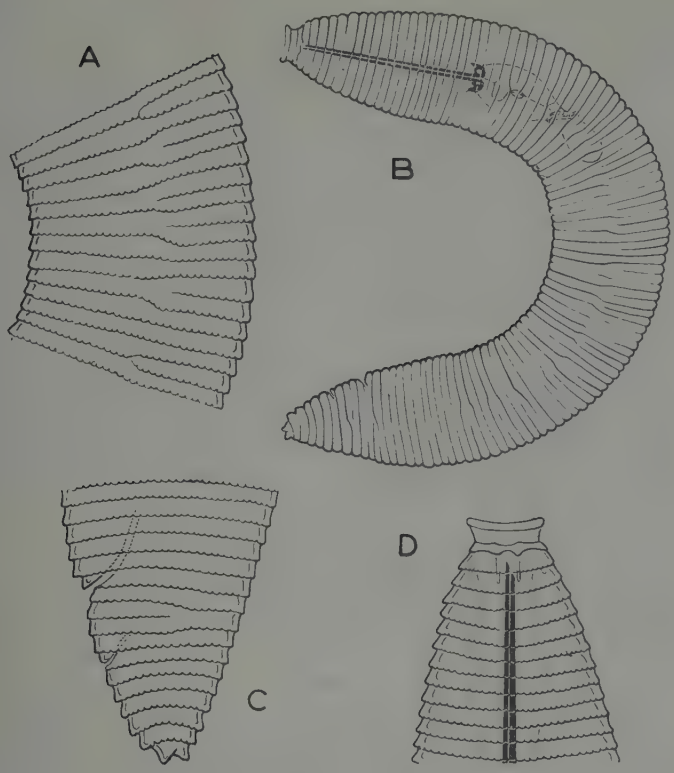

Planche 2: *Criconema limitaneum* n. sp.: A: détail de l'ornementation au milieu du corps. B: vue de l'animal en entier. C: partie postvulvaire. D: détail des anneaux antérieurs.
(Gross.: B × 550. A, C, D × 1100)

ment et 96-111 dorsalement. Le nombre d'anastomoses est très élevé: de 19 à 22. Les anneaux sont ornés sur leur bord postérieur de petites indentations régulières surtout nettes à la partie postérieure du corps. Vulve située à 11 anneaux de l'extrémité postérieure. Partie postvulvaire conique, dernier anneau présentant 1 ou 2 pointes.

Mâle: inconnu.

Holotype: Tube N° 39. Laboratoire de Nématologie I.D.E.R.T. Abidjan; Côte d'Ivoire.

Lieu type: au voisinage de racines de *Cinchona succirubra* Pav., à Seredou, Guinée.

Diagnose: *Criconema limitaneum* n. sp. diffère de toutes les espéces connues du genre *Criconema* par l'ornementation continue et très peu prononcée de ses anneaux. Cette ornementation ressemble beaucoup plus à celle que l'on observe chez les individus juvéniles de certaines espéces de *Criconemoides* (*C. xenoplax* Raski 1952 et *C. mutabile* Taylor 1936 notamment) qu'à celle, spiniforme, qui est de règle dans le genre *Criconema*. Aussi pouvait-on se demander si l'on ne devait pas considérer cette espèce comme un *Criconemoides*. Cependant la structure de la partie antérieure avec son premier anneau en coupe profonde est tout à fait différente de celle des *Criconemoides* chez lesquels le premier anneau diffère des suivants par sa réduction en épaisseur. Par contre la forme cupulée du premier anneau se retrouve chez d'autres *Criconema*: *C. squamosum* (Cobb 1913), Taylor 1936 et *C. cobbi* (Micoletzky 1925) Taylor 1936 en particulier. Nous considérons donc l'espèce décrite comme appartenant au genre *Criconema*: il n'en reste pas moins qu'elle se situe à la limite de ce genre et de *Criconemoides* d'où le nom spécifique qui lui a été accordé.

Hemicriconemoides cocophilus (Loos 1949), Chitwood 1957

Les femelles de cette espèce, observées au voisinage de racines d'ananas à Akoupé (Côte d'Ivoire), correspondent parfaitement à la description originale.

SUMMARY

New Criconematidae of the intertropical zone (Nematoda: Tylenchida)

Descriptions and figures are given of three new species: *Criconemoides onoense* n. sp., *Criconemoides ferniae* n. sp. and *Criconema limitaneum* n. sp. Details are also given on the anterior part of *Criconemoides citri* Steiner 1949.

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THREE NEW SPECIES OF THE GENUS *TYLENCHORHYNCHUS (NEMATODA:* TYLENCHIDA)

BY

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Soil samples processed during the past two years at Auburn, Alabama, have contained numerous species of the genus *Tylenchorhynchus* of which three have been determined as being undescribed. The descriptions of the new species, *T. acti*, n. sp., *T. brevicaudatus*, n. sp., and *T. ewingi* n. sp., are as follows:

Tylenchorhynchus acti new species

(Fig. 1, D and E)

Dimensions. — 5 ♀♀ L = 0.65-0.71 mm. a = 30.2-36.2; b = 4.6-4.9; c = 14.5-16.1; V = 55.2-56.9%. Stylet 16.5-17.3 μ long.

Female (*holotype*) L = 0.66 mm. a = 30.2; b = 4.6; c = 14.7; V = 56.4%. Body cylindrical, tapering at both ends. Lip region bluntly rounded, set off from body contour by slight constriction, bearing 8 annules. Labial sclerotization inconspicuous. Stylet 16.5 microns long, with well-developed basal knobs. Orifice of dorsal esophageal gland about 3.5 microns behind spear base. Excretory pore opens at base of isthmus. Hemizonid one annule anterior to excretory pore. Spermathecae and spermatozoa not seen. Tail conoid, bearing 42 annules, with terminus slightly enlarged, not striated. Length of tail about 3 × anal body diameter. Lateral field with 5 incisures, the middle incisure terminating at the phasmid. Phasmids conspicuous, opening at middle of tail.

Male. — Not known.

Holotype. — Female collected September, 1957, by N. A. Minton, Type Slide No. 1, Alabama Polytechnic Institute Type Slide Collection.

Paratypes. — Same data as holotype.

Type host. — Soil about roots of okra, *Hibiscus esculentus* L.

Type locality. — Home garden of E. C. Sloan, Blountsville, Alabama.

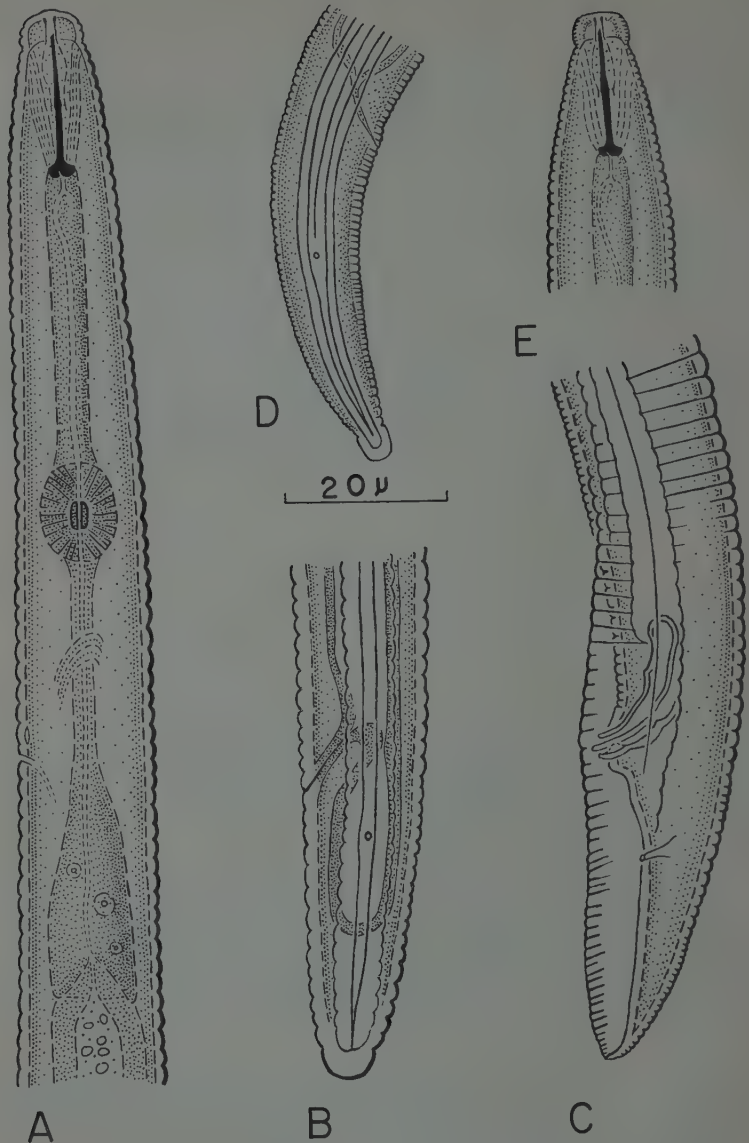

Fig. 1. *Tylenchorhynchus ewingi*: A-C; A, anterior region of female; B, female tail; C, male tail.
T. acti: D and E; D, female tail; E, female head.

Diagnosis. — *Tylenchorhynchus acti* keys to *T. capitatus* Allen, 1955 according to Allen (1955), but differs from it by having the lip region only slightly set off from the body contour and by having a longer tail bearing more annules ending with a characteristic terminus.

The specific name of *T. acti* n. sp., is composed of the initials of Dr. A. C. Tarjan to whom the author is grateful for having introduced him to the fascinating science of nematology and for his never ending patience, inspiration, and encouragement during the early years of his training.

Tylenchorhynchus brevicaudatus new species

(Fig. 2, A — C)

Dimensions. — ♂ ♀♀ L = 1.28-1.37 mm. a = 33.7-35.8; b = 6.7-6.9; c = 33.5-44; V = 57.5-58.6%. Stylet 37-38.4 μ long.
4 ♂♂ L = 0.99-1.17 mm. a = 33.6-36.6; b = 5.5-6.1; c = 29.4-30.8 Stylet 34.6-37 μ long.

Female (holotype). L = 1.33 mm. a = 33.7; b = 6.8; c = 38.4; V = 58.6%. Body cylindrical, tapering at anterior end. Lip region bluntly conoid, continuous with body contour, bearing 8 annules. Labial framework heavily sclerotized, with lateral margins extending posteriorly 1 or 2 body annules. Stylet massive, 37 microns long, with large rounded knobs, the anterior margins slightly directed forward. Orifice of dorsal esophageal gland about 4 microns behind spear base. Excretory pore opens at about middle of basal esophageal bulb. Spermathecae present containing spherical spermatozoa. Tail dorsally convex-conoid, terminus blunt. Length of tail approximately equal to anal body diameter. Annulation of cuticle extends around terminus of tail. Lateral field with 4 incisures. Cuticle coarsely striated. Phasmids prominent, opposite anal opening.

Male (allotype). — L = 1.12 mm. a — 33.6; b = 6; c = 29.4. Stylet 37 μ long. Similar to female. Bursa envelops tail. Spicules tylenchoid. Gubernaculum simple, slightly curved.

Holotype. — Female collected August, 1957, by A. A. Foster, Type Slide No. 2, Alabama Polytechnic Institute Type Slide Collection.

Allotype. — Same data as holotype, Type Slide No. 3, Alabama Polytechnic Institute type Slide Collection.

Paratypes. — Two females, three males, same data as holotype.

Type host. - Soil about roots of Quaking Aspen, *Populus tremuloides* Michx.

Type locality. — Aspen forest, Farmington Flats Campgrounds, Wasatch National Forest, near Farmington Flats, Utah. Altitude 7500 feet.

Diagnosis. — *Tylenchorhynchus brevicaudatus* keys to *T. magnicauda* (Thorne) according to ALLEN (1955) but differs from it by the 8 annules on the lip region, the presence of spermathecae containing spermatozoa, the short tail, the more anterior location of the phasmid and by the presence of males.

T. brevicaudatus, n. sp. derives its specific name from its short tail.

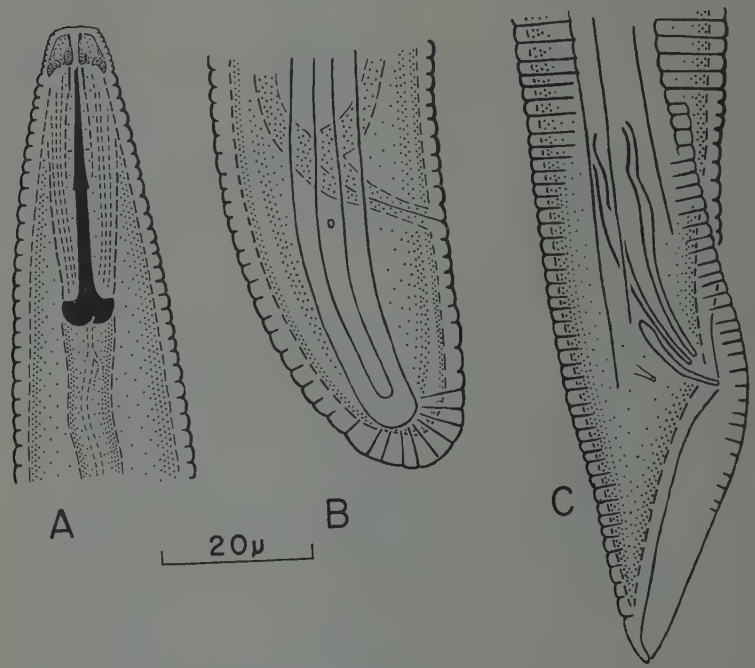

Fig. 2. *Tylenchorhynchus brevicaudatus*: A, female head; B, female tail; C, male tail.

Tylenchorhynchus ewingi new species

(Fig. 1, A-C)

Dimensions. — 10 ♀♀ L = 0.553-0.752 mm. a = 29.5-34.8; b = 4.25-5.5; c = 12.6-15.7; V = 52.7-59.2% Stylet 18.1-20 μ long.
5 ♂♂ L = 0.535-0.629 mm. a = 26.9-32.9; b = 4.57-5.2; c = 13.5-14.8 Stylet 18-19 μ long.

Female (holotype). — L = 0.638 mm. a = 31.4; b = 5.0; c = 14.8; V = 56.9% Body cylindrical, tapering at both ends. Lip region hemispherical, more or less continuous with body contour, bearing 3 annules. Labial sclerotization inconspicuous. Stylet 19 microns long, with well-developed rounded basal knobs. Orifice of dorsal esophageal gland about 2.5 microns behind spear base. Esophageal glands slightly overlapping intestine. Excretory pore opens at level of base of isthmus. Hemizonid one annule anterior to excretory pore. Spermathecae containing spermatozoa present. Tail tapering, conoid, bearing 15 annules, terminus hemispherical, smooth, not striated. Cuticular striations coarse becoming coarser at exterminities. Lateral field with 4 incisures. Phasmids prominent, opening slightly anterior to middle of tail. Postanal intestinal sac present.

Male (allotype). — L = 0.595 mm. a = 31.1; b = 4.7; c = 14.6. Stylet 19 microns long. Similar to female. Phasmids slightly anterior to middle of tail. Bursa envelops tail. Spicules tylenchoid. Gubernaculum large, slightly curved.

Holotype. — Female collected August, 1956, by B. E. Hopper and S. A. Ewing, Type Slide No. 4, Alabama Polytechnic Institute Type Slide Collection.

Allotype. — Same data as holotype, Type Slide No. 5, Alabama Polytechnic Institute Type Slide Collection.

Paratypes. — Same data as holotype.

Type host. — Slash pine(*Pinus elliottii* Engelm.)

Type locality. — Southwest Louisiana Forest Nursery, Oberlin, Louisiana.

Diagnosis. — *Tylenchorhynchus ewingi* is most closely related to *T nudus* Allen, 1955 and is also somewhat similar to *T. martini* Fielding, 1956. It differs from both in that females possess well-developed spermathecae containing spermatozoa. It differs from *T. nudus* by having 3 (4) annules on the lip region as compared to 2 (3?) for *T. nudus*. Also the tail is slightly conoid rather than slightly clavate. The male tail of *T. ewingi* is shorter than that of *T. nudus* (36-46 μ compared to 50 μ). The males also have differences in the gubernaculum (Compare Fig. 1 c with Allen, 1955 Fig. 3 h).

Specimens of this species were found in soil samples from forest nurseries in Oberlin and Sibley, Louisiana, and Newton, Texas.

T. ewingi, n. sp., is named in honor of S. A. Ewing to whom the author wishes to express his appreciation for the assistance rendered during the summer of 1956 and who first recovered this species from soil samples collected at the type locality.

OBSERVATIONS OF THE PATHOGENIC NATURE OF TYLENCHORHYNCHUS EWINGI

In the summer of 1956, *Tylenchorhynchus ewingi* was first found associated with dying pine seedlings at the Southwest Louisiana Forest Tree Nursery, Oberlin, Louisiana [1]) (HOPPER, 1958). Areas containing chlorotic seedlings were evident in several locations in the nursery. This chlorosis, thought at the time to be "fertilizer burn", was first noticed in the 1954 growing season and, according to the nurseryman, had been getting progressively worse. Similar disease symptoms, but more severe, were observed at the old Gaylord Container Nursery site which was located at Morgantown, Mississippi. Here the causal agent was *T. claytoni*. In the 1956 growing season, approximately 40% of one block exhibited the chlorotic seedlings associated with nematode injury.

Above ground symptoms ranged from green-, yellow-, and brown-colored seedlings to dead seedlings. In some areas, this entire array of symptoms was observed from the border of the infested area to its center. Examination of the underground portions of affected stock revealed a reduced root system with very few small roots and no mycorrhiza. This root injury was similar to the "stubby-root" condition of many plants caused by *Trichodorus christiei*. As in "stubby-root", *Tylenchorhynchus ewingi* appeared to attack the root tips devitalizing the growing points. Additional roots were formed behind the affected area and these in turn appeared to be similarly affected by the nematode. The resulting root system was composed of a large tap root, usually with a few laterals bearing clusters of short stubby roots.

T. ewingi populations were lowest in the most severely affected areas in soil samples taken late in the growing season. The highest numbers of the nematode were found associated with the margins of the affected areas and in various other locations within the nursery. In the first instance, the nematode probably was eliminated by destroying the food supply. It is possible that the affected areas were places where the initial nematode population levels were so high that the young seedlings were unable to survive the severe injury. The sites of the highest nematode populations may have been the areas of low initial nematode population. Seedlings growing in these areas tolerated the low populations and therefore showed light injury. These seedlings provided a situation favorable for increase of the nematode later in the season.

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1) *Tylenchorhynchus ewingi* was found while the author was employed by the Southeastern Forest Experiment Station of the U. S. Forest Service.

Population levels may become high enough that economic losses occur. The Gaylord Nursery is considered an example of this type of nematode build-up.

To provide evidence of the pathogenic nature of *T. ewingi,* infested soil was transported to the Nematology Laboratory at Auburn, Alabama, and young pine seedlings transplanted in the soil. Although top symptoms failed to develop, the root systems were severely reduced after a year's growth. The lack of top symptoms may be explained by the fact that the seedlings were transplanted and were not grown from seed in the infested soil. Also, daily watering might have helped alleviate the effect of the root injury on the shoot. These seedlings would have been culled if graded for quality.

Examination of the soil at this time revealed few *T. ewingi* because of the nearly complete absence of root areas suitable for parasitism. Incubation of the root systems produced numerous *T. ewingi,* all of which were larval forms. Apparently this nematode is endoparasitic in at least one stage of its life cycle. Whether it can complete its life cycle in pine roots is still unknown.

In addition to the type locality, *T. ewingi* was found associated with injury to pine seedlings at the Northwest Louisiana Forest Tree Nursery, Sibley, Louisiana, and a small population has been uncovered at the Stillman Nursery near Newton, Texas.

The author wishes to express his appreciation to Dr. W. H. Greenleaf, Horticulture Department, Alabama Polytechnic Institute, Auburn, Alabama, for translation of the summary into German.

ZUSAMMENFASSUNG

Drei neue Arten der Gattung Tylenchorhynchus (*Nematoda: Tylenchida*)

Drei neue Arten der Gattung *Tylenchorhynchus,* nämlich *T. acti,* n. sp., *T. brevicaudatus,* n. sp. und *T. ewingi,* n. sp., werden beschrieben. *T. acti* n. sp. unterscheidet sich von *T. acutus* und von *T. capitatus* dadurch, dass die labiale Region mit der Körperkontur kontinuierlich ist. *T. brevicaudatus* n. sp. besitzt gegenüber von *T. magnicauda* einen kürzeren Schwanz und auch eine Spermatheca. *T. ewingi,* n. sp. hat im Gegensatz zu *T. nudus* im weiblichen Geschlecht einen stumpfen kegelförmigen Schwanz, im männlichen Geschlecht einen kürzeren Schwanz. Ausserdem gibt es noch mehrere Unterschiede zwischen den accessorischen Stücken der Männchen der beiden letztgenannten Arten.

Obgleich die Pathogenität von *T. ewingi* noch nicht wissenschaftlich bewiesen werden ist, liegen doch genügend Tatsachen vor, die seine Rolle als Krankheitserreger bei Kiefern wahrscheinlich machen.

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DEVELOPMENT OF THE ROOT-KNOT NEMATODES *MELOIDOGYNE JAVANICA* (TREUB) AND *MELOIDOGYNE HAPLA* CHITWOOD IN THE TOMATO

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Although members of the genus *Meloidogyne* have been studied extensively, notably by NAGAKURA (1930), TYLER (1933), CHRISTIE & COBB (1941), CHITWOOD (1949) and ELSEA (1951), there is little information available on the times required for the development of each stage of the life cycle and on the morphology of these stages.

NAGAKURA (1930) described three moults within the host plant of which the second was illustrated by diagrams and photographs.

CHRISTIE & COBB (1941) disagreed with NAGAKURA and stated that instead of being spaced at intervals, the second, third and fourth moults all occurred within a short time of each other so that there was no third stage larva and, in the female, the fourth stage was largely theoretical.

As all this work was carried out before CHITWOOD's (1949) reclassification of *Heterodera marioni*, the actual species that these investigators used is unknown. More recently, TARJAN (1952) studied the growth of five species of *Meloidogyne* but data on the occurrence of moults were not obtained.

The present work is an attempt to clarify the developmental morphology of the root-knot nematode using single known species so that studies on the physiology of growth and development in these species may be undertaken.

MATERIALS AND METHODS

Seeds of the Pan america variety of tomato, *Lycopersicon esculentum* Mill., which is susceptible to the root-knot nematode, were germinated

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in either moist sterile sand, filter paper or perlite (a volcanic alumina silicate). Seedlings obtained by these methods were infected with second stage larvae of *Meloidogyne javanica* (Treub), freshly hatched from egg masses which had been removed from females identified previously by microscopic examination of their perineal patterns (Taylor et al., 1955), or by larvae of *Meloidogyne hapla* Chitwood, obtained from egg sacs on the roots of peanut plants. It was possible to obtain large numbers of freshly hatched larvae by storing the egg sacs in 0.3 M NaCl (Dropkin & Martin 1957) and transferring them to distilled water the day before infection. Seedlings were infected either by pipetting larvae around the root tips on 0.6% agar in petri dishes, or more effectively by planting seedlings into perlite containing infective larvae.

Seedlings were exposed to infection for periods of either one or two days, after which they were removed, washed gently to remove any larvae which had not already penetrated and transplanted into perlite in waxed paper cups. These plants were regularly watered with a nutrient solution (Hewitt, 1952).

Experiments were carried out at temperatures of either 20°C, 25°C or, most effectively, at shade temperatures in the open. Recordings were obtained by means of a thermograph. Every twenty-four hours, two seedlings were removed and their roots were gently washed, dried lightly on filter paper and fixed in either T.A.F. (Courtney, Polley & Miller, 1955) at 55°C, room temperature or 8°C or 1% buffered osmium tetroxide (Palade, 1951) at 8°C.

Roots fixed in T.A.F. were subjected to the cotton blue-lactophenol staining technique (Goodey, 1951), transferred to lactophenol and eventually the nematodes were dissected free from the roots and mounted in glycerine by the method of Baker (1953). Roots fixed in 1% buffered osmium tetroxide (periods of from 15-30 minutes in this fixative were found to be adequate) were washed in distilled water for two hours and then dried lightly on filter paper and transferred to T.A.F. The nematodes were dissected free from the roots and mounted in this medium.

Fixation in 1% buffered osmium tetroxide at 8°C was found to be the more suitable because the nematodes were stained differentially in the plant tissue and could easily be seen even in the early stages of development. In this way fixation and staining were combined in one operation. Also it was found that, in some cases, the cotton blue-lactophenol staining technique gave rise to cuticular distortion of the

Figure 1. Head region of a moulting female of *Meloidogyne javanica* showing the three shed cuticles.

nematodes. This might have been due to the fact that roots stained in this manner were allowed to remain in lactophenol for several days before dissecting out the nematodes. The cotton blue technique, however, gave better results for the staining of certain internal structures such as the nuclei of the oesophageal glands.

A growth curve for *M. javanica* females was obtained by drawing the outline of the nematodes on to squared paper using a camera lucida, cutting them out and weighing these cut-out areas to the nearest tenth of a milligramme; a 100 μ square of similar paper, obtained by drawing this area by the above method using a micrometer slide, was weighed and this weight was divided into the weigths of the averaged daily larval cut-outs. In this manner the cross sectional area of the nematodes in square microns was obtained. The average number of nematodes measured per day was eight.

Developing *M. javanica* males were observed in a modification of the perfusion chamber described by PAUL (1957). These larvae developed in glass distilled water and the perfusion chamber was washed out with this twice daily. An antibiotic, 0.05% aureomycin in buffer at pH 7.8 was used initially in these experiments but this was discontinued as bacterial contamination was not very great and did not appear to influence the eventual emergence of the nematode. However, antibiotics may be necessary in future, more critical experiments under higher magnifications.

Larvae in the middle of their first parasitic moult were dissected from infected tomato roots under a stereo-microscope, removed by capillary pipette and placed in the perfusion chamber. Development took place at room temperature and the mean temperature for the week during the particular *in vitro* experiment described below was carried out was 22°C.

RESULTS

Growth of M. javanica

Preliminary examination of hundreds of specimens of *M. javanica* of various ages revealed several larvae in the process of completion of the fourth moult (Fig. 1). It can be seen that both the third and fourth moults occur within the shed cuticle of the second moult as described by CHRISTIE & COBB (1941). Thus the energy necessary for the completion of the third and fourth moults must be obtained by the animal before the second moult because it is unable to feed on its host from the

start of the second moult to the completion of the fourth moult. This means that during the three parasitic moults there is no growth and probably a slight decrease in the size of the parasite. If this is true one would expect to find in a growth curve a plateau or possibly a slight decrease in growth corresponding with the period during which the three moults take place.

Initial experiments carried out at 20°C in a refrigerated constant temperature room near a window did not give significant results because of low infections, unhealthy plants and variations in the rates of growth of the nematodes. At this temperature the onset of moulting was first observed on the twenty-third day. No results were obtained at a temperature of 25°C because of wilting of the host plants. This was probably due to sub-optimum light intensities (see Discussion).

Satisfactory rates of infection were obtained by planting freshly germinated seeds into infected perlite as previously described. These plants were grown under typical summer shade conditions for up to six weeks at temperatures ranging from a nightly minimum of 52°F to a daily maximum of 105°F.

Temperatures fluctuated evenly between these two extremes and remained typical for midsummer. All plants grown under these conditions were healthy and showed a uniform rate of growth and the nematode parasites removed from their roots also showed very little variation in size amongst similarly aged specimens.

The growth curve obtained under these conditions (Fig. 2) was a typical sigmoid curve with the postulated plateau occurring at the onset of moulting on the fourteenth day and extending until the nineteenth day, when moulting was completed and the young adult with its newly formed stylet started to feed actively and grew rapidly. Infective second stage larvae appeared to decrease slightly in size after root penetration.

It is possible that the energy involved in penetration and movement into the root come from food reserves, diminution of which could cause a slight shrinkage of the nematode.

Egg sac formation was first observed on the twenty-seventh day and egg laying commenced two days later. At this stage the female attained almost maximum size; further growth of the nematode itself was slight but the egg sac increased in size and, usually, became larger than the nematode which produced it.

The growth curve data were subjected to statistical analysis. The means and variances within days were calculated. The variances tended to increase with the means except during the moulting period, when

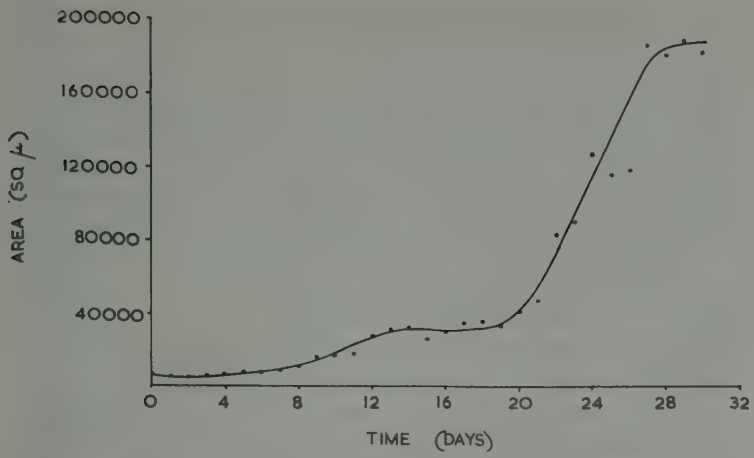

Figure 2. Growth curve for females of *Meloidogyne javanica.*

the means and the variances stayed more or less constant indicating a cessation of growth during this period.

A quadratic curve was fitted to the data from days 0 - 4 and was found to give a significant fit, indicating that the initial decrease in weight is a real effect.

A logarithmic transformaton of the data from days 5-13 made the trend approximately linear. A straight line was then fitted to the log weights and the value of the regression coefficient b was 0.076 with a standard error of $\pm$ 0.006 on 7 degrees of freedom. Thus b is very highly significant. The data for days 14-18 were treated similarly and the value of b was 0.020 with a standard error of $\pm$ 0.017 on 3 degrees of freedom. This is not significantly different from zero, indicating that there is a flattening in the growth curve during this period.

The data for days 19-27 were analysed and the value for b was found to be 0.092 with a standard error of $\pm$ 0.007 on 7 degrees of freedom, again highly significant.

Thus statistical analysis of the 245 specimens included in the growth curve (Fig. 2) supports the shape of this curve and the deductions drawn from it.

The infective larvae migrated a short distance in the cortex of the host plant after penetration and remained in the cortex with their heads near the vascular system. Growth was slow at first. In the first eight days the parasite only doubled its cross sectional area but in the five

days that followed this the cross sectional area was almost trebled. The larva became thicker and just before the second moult had a characteristic spiked tail. Its stylet was 14 μ long throughout this period. During the three moults which followed each other between the fourteenth and nineteenth days there was no increase in size. On the fourteenth day three specimens underwent the second (first parasitic) moult, one started to moult and the remaining three specimens had not started to moult. On the fifteenth day all specimens were in the process of moulting and were undergoing the fourth moult. Between the sixteenth and nineteenth days all specimens were either in the final moult or had completed it and were adults.

Normally, under optimum conditions such as these, parasitic moulting seems to take place during a period of about three days. The first moult appears to take about one day and is followed rapidly by the second and third moults which do not involve the structural breakdown associated with the first moult. The specimens appear to remain surrounded by the moulted cuticles for two or three days while considerable resynthesis of internal structure takes place. During the first moult the anterior part of the stylet is shed and the posterior part breaks down; there is no stylet in either the third or fourth stage larvae. After completion of the final moult a new and somewhat larger stylet is formed. This is the adult stylet. In the female it is 17.5 μ long and is much thicker than the larval stylet. Its knobs are 4 μ across compared with the 2 μ width of the larval stylet knobs. Between the twentieth and twenty-seventh days growth is extremely rapid. The nematodes more than quadruple their cross sectional area. During this time and the time of egg sac formation and egg laying the parasites must exert their greatest strain on the host plant.

Although the growth curve (Fig. 2) includes only specimens up to an age of thirty days, specimens were measured up to an age of forty days. At this age the average cross sectional area of the specimens measured was slightly over 200,000 sq μ so that the rate of growth of the nematodes between the thirtieth and fortieth days was slight. However, egg sac growth increased rapidly during this period as did the rate of egg laying.

The regions of the oesophageal gland and excretory pore and duct were examined with care throughout larval development. It was thought that possible changes in these structures during development might furnish some further evidence for the recent interesting endocrine hypothesis of nematode ecdysis postulated by ROGERS & SOMMERVILLE

(1957). Also, the "exsheathing fluid or its precursor" (SOMMERVILLE, 1957) is released from a region near the excretory cell in infective larvae of *Trichostrongylus axei*. It was possible to observe the excretory pore and duct in several larvae before the onset of moulting. No excretory cell or renette was observed. The excretory system in these larvae opens to the exterior near the basal region of the median bulb. The duct may be followed backwards for some distance before it becomes hidden beneath the contents of the gut. The excretory system was most

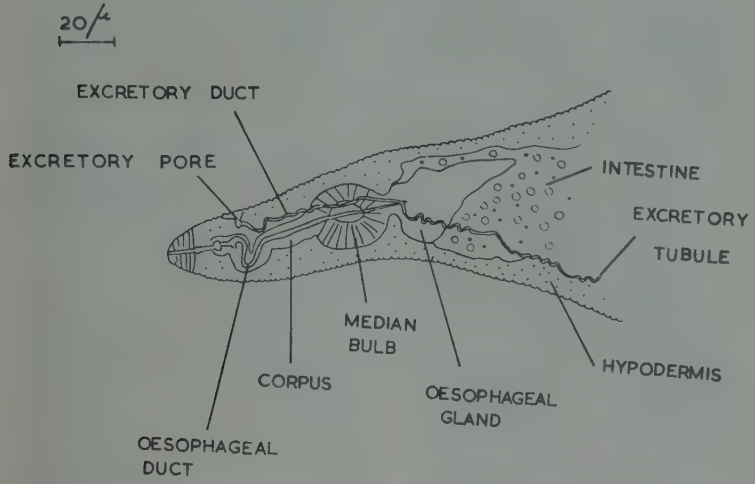

Figure 3. Head and neck region of a young adult female *Meloidogyne javanica* — showing the excretory pore and tubule.

clearly seen in young adult female specimens (Fig. 3) which had just completed the final moult. The relative position of the excretory pore changed during moulting and was then located anteriorly about twenty-five microns in front of the anterior portion of the median bulb. The excretory duct ran back across the median bulb to the surface of the oesophageal gland and then bent out of focus as it moved towards the hypodermis and passed from the neck region into the body of the nematode. It could be traced back for some 70 μ where it appeared to merge with the hypodermis.

It was not possible to obtain a correlation between the size of the oesophageal gland and the onset of moulting. The gland did appear to be larger just before moulting commenced but it was not an easy structure to stain especially with osmium tetroxide and so results such as

these on gross size must be interpreted with a great deal of caution. Obvious changes did, however, occur in the hypodermis and this part of the nematode became much thicker and was much more clearly demarcated just before moulting commenced. This thickening of the hypodermis was first noticed on the twelfth day in specimens observed in the growth curve experiments.

Growth of M. hapla

Similarly, aged specimens of *Meloidogyne hapla* grown under similar conditions and at the same time as *M. javanica* showed, rather surprisingly, a similar rate of growth. There were not enough specimens per day to make a suitable growth curve but there were enough to enable comparisons with *M. javanica* to be made. *M. hapla* is generally considered to have its optimum growth rate under somewhat cooler conditions. Higher temperatures might affect the ability of the second stage larva of this species to penetrate rather than influence growth rates once it is within the host.

Observations of larvae of M. javanica in a perfusion chamber

Because the third and fourth moults occur within the shed cuticle of the second moult, the larval nematode is unable to feed and is dependent on stored energy for these moults and, in the case of the male, for the synthesis of adult organs.

Advantage has been taken of these peculiarities to examine intact freshly dissected *M. javanica* larvae in a modification of the perfusion chamber described by PAUL (1957). Only males have been observed to develop *in vitro* under these conditions. During the first day in a typical specimen (Fig. 4. 1) there was slight movement in the head region, the hypodermis was very thick and contrasted with the dark coloured gut which it surrounded. On the morning of the second day (Fig. 4. 2) there was a great deal more activity; the larva had contracted away from the shed cuticle of the first parasitic moult and had slightly increased in length and decreased in width. It was able to withdraw its head for brief periods and so demonstrate the shed cuticle of the second parasitic moult. This was seen more clearly at a later stage when the head flexure was pronounced (Fig. 4. 4 & 5). During the afternoon of the second day growth proceeded at a remarkable rate (Fig. 4. 3, 4 & 5); moving vigorously, the larva doubled its length within five hours. On the third day there was no evidence of further growth nor of internal organ formation. However, by the fourth day the stylet

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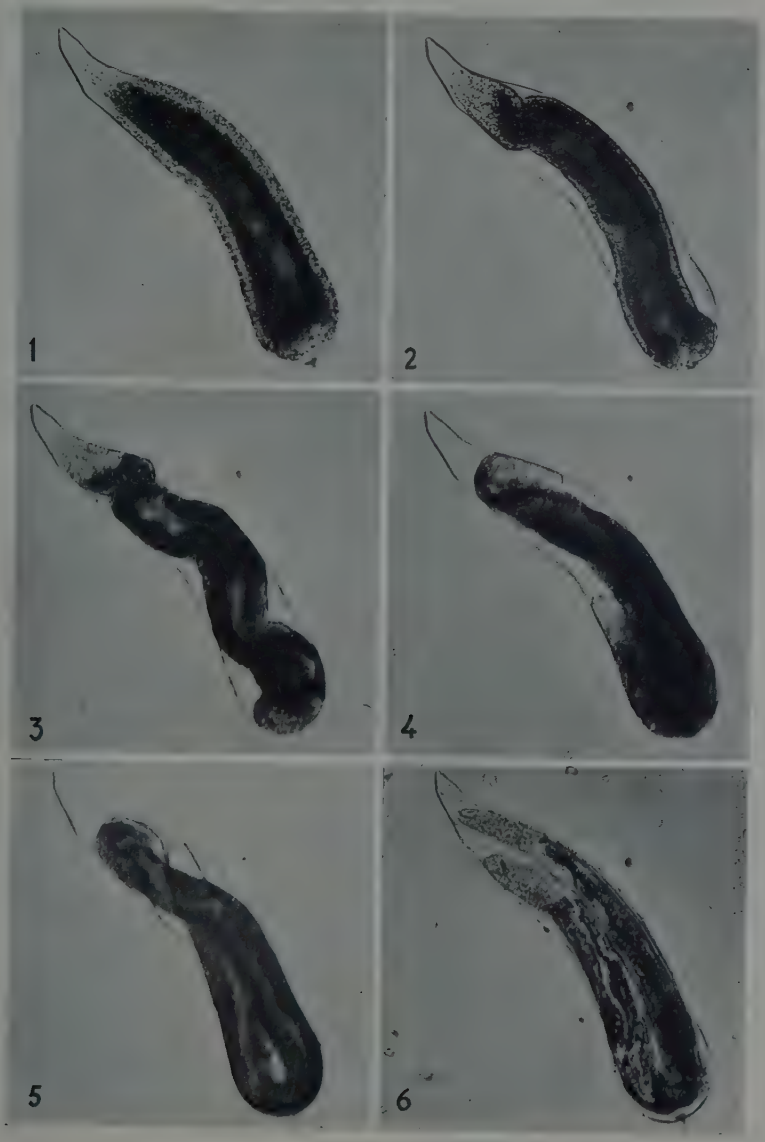

Figure 4. Development of a male *Meloidogyne javanica in vitro*. 1. Taken at 12.30 pm on the first day. — 2. 10 am on the second day. — 3. 2 pm on the second day. — 4. 3.30 pm on the second day. — 5. 6.45 pm on the second day. — 6. 9 am on the fifth day.

was formed but was not functional and spicule formation had started. Spicule formation was completed by the fifth day (Fig. 4. 6) and there was some evidence of the final moult in the head and tail region.

During the afternoon of the fifth day the larva passed into a quiescent state which persisted over the sixth day and was obviously associated with internal reorganization because on the seventh day the larva was active again and organ formation appeared to be complete. The newly formed adult used its stylet to puncture the outermost cuticle and emerged for two-thirds of its length. The tail region remained enclosed in the shed cuticle, presumably because the nematode could not get sufficient purchase on the glass to pull itself free. The adult male was removed from the perfusion chamber, fixed in cold T.A.F. and mounted and observed under higher magnification. It was not stunted and had normal dimensions.

DISCUSSION

The failure to grow tomato plants satisfactorily at a constant temperature of 25°C in these experiments was probably due to sub-optimum light intensities. WENT (1945) has shown that there is an important correlation between the amount of light received by tomatoes and their optimal dark temperatures. The optimal night temperature falls off very rapidly with decreasing total illumination. Temperatures above the night optimum give less growth due to insufficient translocation of sugars from the leaves at these temperatures.

There has not been a great deal of work done on growth in nematodes and few growth curves have been plotted, especially for phytoparasitic nematodes. The growth of several species of the genus *Meloidogyne* has been plotted in one curve by TARJAN (1952) who plotted maximum width against age in days. This curve, unlike the one depicted in this paper, contained several steps. The methods involved in obtaining TARJAN's curve are open to some criticisms. Firstly, the exact age of the nematodes was unknown because the host plants were not removed from the infective soil and only the oldest established infections were measured. Secondly, the nematodes were not dissected out of the roots but were measured *in situ* by a staining technique which precluded observations on the occurrence of moults. Thirdly, the growth curve depicted by TARJAN is a mean curve representing five different species.

The nematode measurements obtained by the methods described in this paper are two-dimensional. Measurements of volume or weighing

would give better representation of the rate of growth. The size of these creatures predisposes against these techniques, however. Three dimensional measurements, if feasible, would be expected to steepen the post moult part of the curve.

The majority of nematodes grow in a series of steps as they feed, grow and moult. The genus *Meloidogyne* is peculiar in that, after entering the plant and commencing to feed, it grows and increases considerably in size before the onset of moulting. After moulting the nematode grows rapidly and increases in size. The rapid increase in size which is not interrupted by moulting must necessitate either a stretching of the cuticle or its active growth before and after moulting. If pre- or post-moult increase in size is achieved through cuticle elasticity, the cuticle should become thinner as the nematode increases in size. This is not the case and there appears to be further evidence for the hypothesis originally put forward for zooparasitic nematodes (Bird, 1957) that the cuticle is a living structure which is in a state of constant metabolic activity and not just an inert exoskeleton. The increase in thickness of the hypodermis just before moulting points towards the probable rôle of this structure in cuticle secretion.

Nagakura (1930) was unable to detect an excretory system in adult female root-knot nematodes and concluded that this problem required further investigation. Christie & Cobb (1941) included an excretory pore and part of the duct in their drawing and placed the opening opposite the median bulb. The parts of the excretory system observed in these investigations resemble those drawn from *M. javanica* by Chitwood (1949). However, they are much simpler than the complex system of unlabelled entwining tubules depicted by Chitwood. Finer structures and tubules have been observed in some specimens but their connections with each other have not been resolved clearly enough to justify their inclusion.

It has been suggested by Elsea (1951) that in *M. hapla* the excretory canal is connected with an anterior tubular structure which in turn connects with a posterior system of canals and tubules which he has demonstrated in the hypodermis by means of sections. He states "—— that it would seem that all are connected in some way with removal or concentration (or both) of a colloidal waste secretion which is collected in the tubules and eventually eliminated from the body through the excretory pore". There is as yet no physiological evidence to support Elsea's contentions.

It is probable that the excretory system of nematodes serves several

functions. Its apparent connection with the hypodermis which probably secretes the cuticle, makes it a target for further studies on the mechanism of moulting in these nematodes.

The perfusion technique described in these experiments shows promise. The results obtained are only of a preliminary nature and conclusions from these experiments should be treated with caution. Perfusion methods involving temperature control and greater optical resolutions combined with improved histochemical staining methods and electron microscopic examination of these small nematodes will undoubtedly assist in furthering this type of work.

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ZUSAMMENFASSUNG

Die Entwicklung der Wurzelgallennematoden Meloidogyne javanica (*Treub*) *und* Meloidogyne hapla *Chitwood in der Tomate*

Das Verhalten von *Meloidogyne javanica* und in geringem Umfange auch das von *M. hapla* bei der Häutung wird beschrieben. Eine Wachstumskurve wurde für das Weibchen von *M. javanica* entworfen. Diese Kurve verläuft s-förmig mit einer Höhe, die den Häutungsperioden entspricht. Unter optimalen Wachstumsbedingungen trat die Häutung zuerst am 14. Tag auf. Das letzte Exemplar beendete seine Häutung am 19. Tag. Die Bildung des Eiersacks wurde erstmalig am 27. Tag beobachtet, die Eibildung begann 2 Tage später. Die Stachellänge vor und nach der Häutung und die Lage des Exkretionsporus zeigen deutliche Unterschiede. Eine Dickenzunahme der Hypodermis unmittelbar vor Beginn der Häutung wurde festgestellt.

Die ungewöhnliche Metamorphose des Nematoden wurde benutzt, um die Entwicklung des Männchens von *M. javanica* in einem Durchströmungsversuch *in vitro* zu beobachten.

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THE DEVELOPMENT OF A TECHNIQUE FOR STUDYING THE HOST/PARASITE RELATIONSHIP OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* UNDER CONTROLLED CONDITIONS

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Observations on the nature of resistance in plants to nematodes present a complex picture; much work prior to CHITWOOD's (1949) classification of the genus *Meloidogyne* was apparently contradictory because of confusion of the species involved. Resistance is a term which has been used loosely to cover a wide range of unsuitable host/parasite relationships; for example, potato and tomato, susceptible to potato root eelworm (*Heterodera rostochiensis*) and root knot eelworms (*Meloidogyne* spp.) are "resistant" to beet eelworm (*Heterodera schachtii*) : *Lycopersicon peruvianum*, susceptible to *Meloidogyne arenaria*, is less susceptible to *M. incognita* var. *acrita* and *M. hapla*, and "resistant" to *M. incognita* (TAYLOR & CHITWOOD 1951) : *Heterodera rostochiensis* develops normally on tomato and potato (*Solanum tuberosum*) but rarely on *S. nigrum* or on resistant lines of *S. tuberosum* subsp. *andigena* (DONCASTER 1953; ELLENBY 1954; JONES 1954). Different degrees of resistance also are recognised (CHRISTIE 1949, SASSER & TAYLOR 1952); absolute resistance occurs when no larvae gain entry to the root; few may enter and few or none develop; many may enter and few or none develop; many may enter and many develop.

LIAO & DUNLAP (1950) reported that invasion was arrested in 2-day old *Lycopersicon peruvianum* seedlings when *Meloidogyne* larvae were about half embedded in young rootbuds; some had died in this position. DONCASTER (1953) showed that his population of *H. rostochiensis* larvae invaded roots of *Solanum nigrum* in reduced numbers but failed to develop, degenerating and dying before or soon after the second moult. This happened also with *Meloidogyne* larvae in roots of *Crotalaria retusa* and *C. striata* (PEACOCK 1957). CHRISTIE (1946) reported that *Meloidogyne* larvae entered roots of *Nicotiana megalosiphon* as

readily as they did roots of *N. tabacum*, but only a few individuals reached maturity; in *N. plumbaginifolia* few larvae survived beyond the first moult. In susceptible plants where normal development occurred, the duration of the life-cycle varied in different host species. Christie (1949) pointed out that resistance may not necessarily be of the same nature in all plants.

Many authors have described the histological changes in root tissue of susceptible plants brought about by the presence of developing nematodes. In general these changes involve the formation of giant cells with breakdown of cell walls, distortion of the vascular elements and in the case of root-knot nematodes, proliferation of the parenchyma to form the characteristic galls (Kostoff & Kendall 1930, Christie 1936). Linford (1937) demonstrated photographically the puncturing of cells and injection of saliva by the nematode during feeding, and Dropkin (1954) has correlated gall-size with the number of *Meloidogyne* larvae present, stating that root response is a local one and is related to the amount of stimulation provided by each nematode. In resistant plants, cells surrounding the head of the parasite are necrotic, but giant cells are not generally formed. Williams (1956) reported that in resistant *Solanum tuberosum* subsp. *andigena* plants very few *H. rostochiensis* females developed. Those that did were always associated with the giant cells observed.

Several authors have postulated the synthesis by resistant plants of a chemical which inhibits or neutralises the salivary secretions of the nematode responsible for giant-cell formation (Barrons 1939, Christie 1949, Liao & Dunlap 1950, Bovien 1955); a nematicidal root-diffusate specific against *Pratylenchus* spp. has been reported to occur in roots of *Tagetes patula* and *T. erecta* (Oostenbrink, Kuiper & s'Jacob 1957). The attractiveness of roots to nematodes is also thought to have a chemical basis; the hatching and attraction of *H. rostochiensis* larvae by a root secretion from potato and other plants is well known (O'Brien & Prentice 1930, Triffitt 1930, Todd et al 1947). Gadd & Loos (1941) working with *Anguillulina* (*Pratylenchus*) *pratensis* and *Heterodera marioni* (*Meloidogyne* spp.) and Wieser (1955) with *Meloidogyne* spp. have shown that attractiveness of roots to these nematodes is dependent upon rate of growth of the root, and postulated a chemical attractant synthesised by growing tissue. Wieser (1955, 1956) suggested also that certain parts of the root — the apex and an area behind the region of elongation — were repellent to larvae, and postulated the presence of both attractive and repellent chemicals in the

growing root. ELLENBY (1954b) has reported that under conditions of heavy nematode infestation of a plant, i.e. when food supply might be expected to be short, the sex-ratio of the nematode was upset, so that fewer females and more males were produced. WILLIAMS (1956) suggested that a starvation-induced change in the sex-ratio might explain the lack of mature females in resistant plant tissues.

The following ways in which an unknown chemical in a plant may be responsible for resistance of the plant to nematode attack may now be listed: —

(a) by masking the attractant substance in the root, or by actively repelling the nematode.
(b) by killing the nematode on entry, or retarding its development.
(c) by neutralising the effect of nematode saliva on giant-cell formation.
(d) by changing the composition of the cell-wall, so that nematode saliva is no longer effective, or the cell-wall is impenetrable to the nematode stylet.
(e) by upsetting the sex-ratio of the nematode, either physiologically or by eliminating the females.

A technique was needed whereby the host/parasite relationship could be studied under carefully controlled conditions and by means of which experimentally induced changes in the environment might lead to the development of one or more of these situations.

The difficulty of controlling root growth and of observing eelworms in soil led to experiments with various artificial media, and finally to root cultures in sterile culture solution, which imposed the discipline of asepsis on all stages of the technique.

TECHNIQUE

A pure line of *Meloidogyne incognita* (Kofoid & White) was cultured from a single egg-mass. Each week twelve small tomato plants were inoculated by pipetting a suspension of larvae in water into a pencil-hole in the soil at the base of the plant. Viable egg-masses can be collected from these plants after 4-5 weeks in summer, 7-8 weeks in winter (soil temperatures 25-30°C) and used for experimental work or to perpetuate the culture. Previous work with *Meloidogyne* spp. (PEACOCK 1957) showed that at high soil temperatures, coherent egg-masses at the surface of the root were produced, which could be picked off with forceps or a needle and which commenced hatching immedia-

tely on being placed in clean water. By drawing off hatched larvae daily with a pipette a constant supply of day-old larvae was possible.

Several workers have reported methods for sterilising larvae or eggs of various soil-inhabiting eelworms. DOUGHERTY & CALHOUN (1948) used penicillin, streptomycin, and merthiolate to sterilise larvae of *Rhabditis* spp; MOUNTAIN (1955) used streptomycin sulphate and ultraviolet light on larvae of *Pratylenchus minyus*. LOWNSBERY & LOWNSBERY (1956) described a method of testing the effectiveness of sterilants and reported a partial success with a dihydrostreptomycin sulphate/ethoxyethyl mercury chloride treatment on larvae of *Meloidogyne incognita*. FENWICK (1956) sterilised eggs of *Heterodera rostochiensis* without impairing viability by treatment with hydrogen peroxide; this was the simplest and quickest method, involving treatment for 8 hours, then washing.

Handling of large numbers of aseptic eelworm eggs or larvae is quite difficult and it was felt that surface-sterilising *Meloidogyne* egg-masses would have the advantage of providing an easily transferable sterile inoculum of several hundred larvae. A small number of known antiseptic compounds was tested for high fungi- and bactericidal activity coupled with low nematicidal activity. Results are summarised in Table I. Phenyl mercury acetate, mercuric chloride, and Hibitane diacetate [1]) were satisfactory on both counts; the latter was preferred to the mercury compounds which were liable to be toxic to root cultures and are adsorbed on glassware. Further work with Hibitane diacetate showed that immersion of egg-masses for 15 minutes in a 0.5% solution gave a high degree of asepsis. This was later improved upon by a preliminary washing in 0.1% Cetavlon [2]), so that the final treatment was as follows: —

Wash 5 minutes in 0.1% Cetavlon; decant and rinse in distilled water.

Immerse in 0.5% Hibitane diacetate for 15 minutes. Rinse in sterile distilled water.

Larvae have remained aseptic and active after treatment for up to 5 weeks. Their vigour was undiminished as compared with unsterile larvae of the same age when both were used to infect young tomato plants. A further refinement of technique consists of using egg-masses as young as possible consistent with their containing viable eggs. This avoids internal contamination of empty egg-cases with microorganisms.

Seeds of *Lycopersicon esculentum* (tomato, susceptible) and *L. peru-*

1) Bis [p-chlorophenyldiguanido]-hexane-diacetate.
2) Cetyl trimethylammonium bromide.

TABLE I

Comparison of various compounds as surface-sterilants for Meloidogyne *egg-masses*
Marks in scale 1-10

Chemical	Short contact — 5 min.			Long contact — 2 days		
	Concn. %	Relative Mould growth	Relative Larval hatch	Concn. %	Relative Mould growth	Relative Larval hatch
Control — distilled water	—	1	10	—	0	10
Control — nutrient solution	—	10	3	—	10	0
p-Chlormetaxylenol 3%	50.0	10	8	—	—	—
Terpineol 5% ("Supersan")	100.0	4	10	—	—	—
Sodium borate	—	—	—	0.5	0	7
(Borax)	—	—	—	1.0	0	5
Sodium pentachlorphenate	1.0	4	4	0.1	0	0
	5.0	2	7	0.5	0	0
Phenyl mercury acetate	0.001	2	9	0.0002	0	0
	0.005	0	9	0.0005	0	0
Mercuric chloride	0.005	0	10	—	—	—
	0.05*	0	10	—	—	—
Cetyl pyridinium bromide (Fixanol C)	0.5	8	6	—	—	—
	2.0	8	6	—	—	—
Cetyl trimethyl-ammonium bromide (Cetavlon)	—	—	—	0.5	0	4
	—	—	—	1.0	0	3
Bis- p-chlorophenyl diguanido -hexane diacetate (Hibitane di-acetate)	1.9 (saturation)	0	10**	0.1	0	10
				0.5	0	thick precipitate

* 15 seconds contact only
** much the largest number of larvae hatched

vianum (resistant) were surface sterilised with Hibitane diacetate, and germinated on sterile moist filter paper in petri-dishes. When roots were 10-15 mm long they were cut off and transferred to 100 ml conical flasks each containing 50 ml of White's nutrient solution (WHITE 1943, STREET 1957). All but one of each species were discarded, the selected root after 8 days growth being cut into 10-15 mm pieces and sub-cultured into fresh nutrient. In this way all root material was of the same clonal origin. Sub-culturing was carried out every 8 days and

there were two types of culture — (i) tip cultures, in which the growing point was excised and gave rise to a long root bearing numerous side-buds; this when cut up provided (ii) sector cultures in which the side-buds grew to give a number of tips which were used for experimental work. The pH of the nutrient solution was most important, and when additions were made to it, the solution was checked and adjusted to return it to its natural pH of 4.6 before autoclaving at 15 lbs/sq. in for 5 minutes. The root cultures were kept in the dark or in semi-darkness at a temperature of 27°C. Root transfers were made with a sterile wire loop, the roots having been cut in the flask with sterile scissors (surgeon's ear and throat).

Because larvae cannot invade roots in culture solution (larvae dissipate their energy in swimming and have nothing to "push against") experiments were made with a number of solid and semi-solid media. Sand and moist filter paper were of no use; vermiculite was satisfactory but observation of the root was prevented. Root growth was poor on and in various nutrient agars, and hence larvae were less strongly attracted to the root. Finally, a method was adopted whereby the root-piece was transferred to a suitable substrate for larval penetration for a limited time only, being returned after infection to the culture solution. The most satisfactory substrate was found to be 0.5% nutrient agar shaken in a test-tube and allowed to drain down the walls of the tube leaving a thin agar film on the glass; the root piece was transferred aseptically to this film and an aseptic egg-mass was placed near the growing point. After 24 hours, the root was replaced in fresh culture solution.

RESULTS

It was absolutely essential for success to keep the tubes containing root pieces in a moisture-saturated atmosphere. In *Lycopersicon esculentum* under these conditions root swelling was visible in 24-48 hours, and under optimum culture conditions the whole life-cycle to the production by the female eelworm of viable eggs occupied 33 days. During the 24-hour period in the test-tube, attraction of larvae to the root and invasion of the root could be carefully watched under the microscope. Attraction was to a very closely defined area immediately behind the apical meristem (as described by CHRISTIE 1936) and characteristically a large number of larvae invaded simultaneously through a small puncture — presumably made by the first larva to gain entry. Attraction appeared to be stronger once the root was punctured (cf. LINFORD 1942) and larvae entering the root were disposed like the

petals of a daisy; the size of the hole became enlarged and often the cells surrounding the hole were necrotic. In some root-tips apical dominance diminished and lateral root-buds became active; larvae were then strongly attracted to these laterals and not to the primary apex. Nothing was seen which supported WIESER's theory of repellent areas of root, but when linear growth ceased larvae ceased to be attracted.

To demonstrate the chemical nature of the attraction a technique originally used to demonstrate toxic secretions of fungal hyphae in soil (KERR, 1956) was adapted for laboratory conditions. A sterile piece of cellophane 1 " × 1½" was placed on a shaken agar film in a test tube. A sterile egg-mass was inserted between the glass and the cellophane (i.e. in the agar) and an excised root tip was placed on the top surface of the cellophane. Tubes were kept in a moist chamber and examined after 24 hours, when large numbers of larvae were found clustered over the growing point. As the root-tip grew away, larvae moved with it on the other side of the cellophane, but no penetration occurred until root-tip and larvae reached the edge of the cellophane piece.

Development of parasite in host tissues in culture solution.

It has been stated that the life-cycle occupied 33 days under optimum conditions. These conditions were obtained when a relatively small number of larvae invaded the root-tip, so that the tip was able to continue growing on being returned to the culture solution; root growth was normal, much of it at the liquid/air interface.

Frequently, however, larval invasion of the root-tip occurred in such massive numbers that the tip was destroyed; on being returned to the culture solution some of these roots sank, and made no further growth. Others grew side roots and the site of invasion became a "cul-de-sac". Under these conditions eelworm development varied considerably, but even where roots sank, turned brown, and made no further growth individual females developed to maturity (taking up to 8 or 9 weeks to do so).

Attempts were made to improve growth of roots and/or development of larvae in roots in culture solution after invasion with larvae by the addition to the culture solution of yeast extract 0-100 p.p.m. and casein hydrolysate 0-5000 p.p.m., singly and combined; the effect on larvae was assessed after 26-30 days by staining the roots in boiling lactophenol/acid fuchsin (GOODEY 1949) and observing under high power microscope the degree of development of the individual larvae. Although

in one experiment 100 p.p.m. casein hydrolysate appeared to encourage root and larval development, conclusions drawn from a number of experiments were that larval development at least owed nothing to the casein hydrolysate or yeast extract, and was independent even of the presence or absence of vitamins in the culture solution. Vitamin requirements must be minimal and adequately covered by the "carry-over" in the root piece from the previous passage in vitamin-containing culture solution.

An application of the technique.

Although the main object was to study the effect, on the root/parasite association, of chemical changes in the environment, the first application of the technique was to help define the site of resistance in plants.

Baranyovits (in litt.) in experiments on cross-grafting and approach-grafting resistant solanaceous plants (*Atropa belladonna, Datura stramonium, Hyoscyamus niger, Solanum nigrum* and *S. dulcamara*) onto tomatoes showed that resistance or susceptibility of the plant to *H. rostochiensis* was determined by the rootstock. Forster (1956), cross-grafting *S. nigrum* onto tomato and inoculating with *H. rostochiensis* larvae also showed this to be the case. When she inoculated cross-grafting *S. nigrum* onto tomato and inoculating with *H. rostoloidogyne incognita* larvae, however, she found that ... "In plants with tomato root-systems many females were found, but the total numbers and the percentage of those with eggs were significantly lower on those with *L. peruvianum* scions than on the self-grafted tomatoes". She concluded that whilst a tomato scion did not make a *L. peruvianum* root any less resistant, a *L. peruvianum* scion did make a tomato root less susceptible to *M. incognita*. This suggested that, wherever the "resistance factor" was produced, it was capable of downward movement from stem to root, and it was decided to see whether roots of a resistant plant lost their resistance when the influence of the aerial part of the plant was eliminated.

Twenty aseptic root tips of *L. peruvianum* were taken from clonal material which had been sub-cultured through 27 passages in nutrient solution. The same number of tips of *L. esculentum* were used as controls, and these had been sub-cultured through 33 passages. The roots were exposed to *Meloidogyne incognita* larvae in an agar film for 48 hours and afterwards returned to culture solution where they remained until egg-masses were visible on some of the roots, 5½ weeks later. Concurrently 10 whole plants for each species, growing in soil, were

inoculated with *M. incognita* larvae and grown until egg-masses were seen, in this case 4½ weeks later. Observations on the whole plants were restricted to a final assessment of knots and egg-masses. With excised root tips however it was possible to assess root attractiveness to larvae, the number of roots invaded, and the degree of development of larvae subsequently. Results are summarised in Tables II to IV.

TABLE II

Invasion of plants grown in soil inoculated with Meloidogyne incognita *larvae. Observations after 4½ weeks. Means of ten replicates*

Host species	Knots formed: Mean number per plant	Knots formed: Dimensions	Egg-masses: Number per plant	Egg-masses: No. of eggs per mass	Egg-masses: No. of eggs containing larvae
Lycopersicon esculentum	191	up to 5.0 mm by up to 25.0 mm	estimated 470	557	259
Lycopersicon peruvianum	6	0.5 — 1.0 mm by 0.5 — 1.0 mm	5	232	28

TABLE III

Attractiveness of excised root tips to Meloidogyne incognita *larvae under aseptic conditions. Observations after 44 hours. Means of twenty replicates*

Host species	Linear root increase (mm)	Number of larvae visible*	Number of larvae attracted**	Number of roots already swollen
Lycopersicon esculentum	9.0	43	31	13
Lycopersicon peruvianum	15.7	42	13	0

* This takes no account of larvae which may already have entered the root.
** i.e. clustered upon the root tip.

Lycopersicon peruvianum root tips remained resistant to *Meloidogyne incognita* larvae after 27 passages in culture solution, thus demonstrating that resistance is perpetuated by growing root tissues, and does not depend on translocation of metabolites from the aerial part of the plant. But FORSTER's result suggests that the stem meristems may also in certain cases produce a "resistance factor", some of which may circulate in the plant.

TABLE IV

The same roots as in Table III stained and examined after 5½ weeks in culture solution. Means of twenty replicates

	No. of roots	Number and stage of development* of nematodes seen on staining					
		A	B	C	D	Σ	Total
Lycopersicon esculentum	15	102	135	16	11	15	279
Lycopersicon peruvianum	18	10	4	2	1	4	21

A little or no development.
B thickened forms having hemispherical posterior end with "spike" up to final moult.
C juvenile female (completed final moult, no "spike").
D mature female
E egg-laying female.

* (after CHRISTIE 1946).

Resistance of L. peruvianum *to the parasite.*

In the tests described above, there was no one stage at which the resistance of a root became operative. Larvae were attracted to excised root tips of *L. peruvianum,* though less strongly than to *L. esculentum* root tips. They penetrated *L. peruvianum* root tips, in lesser numbers but in the same way as they did *L. esculentum,* mass invasion occurring commonly at a single entry point. They developed in the root at a slower rate, and only where the root reacted with hypertrophy and giant cell formation; in 12 out of 18 roots there was little or no development and no root swelling. Frequently the points of larval entry became necrotic and subsequently callus outgrowths occurred. In 3 roots degenerating stage A and B larvae were found; few males were seen.

There was never more than one developing female in a swelling — (up to 8 occurred together in *L. esculentum*). This was characteristic also of whole plants grown in soil, where simple knots typically contained one egg-laying female and a number of undeveloped larvae, some of which were dead. This may be tentatively explained by supposing that the resistance of the root can be temporarily overcome by a mass invasion of larvae, but that subsequent development is confined to the strongest or most favourably placed individual from which a resistance-breaking biotype may arise. Or it may be that the biotype already exists in the population, as JONES (1957, 1958) and DUNNETT (1957) have reported for *Heterodera rostochiensis.*

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ZUSAMMENFASSUNG

Die Entwicklung einer Technik zum Studium der Beziehung zwischen Wirt und Parasit beim Wurzelgallennematoden (Meloidogyne incognita) *unter festgelegten Bedingungen*

Wenn Nematodenlarven eine geeignete Wirtspflanze befallen, so reagiert die Pflanze mit einer Reihe von Zellveränderungen. Es wird angenommen, dass diese durch den Speichel der Nematoden hervorgerufen werden. In einem ungeeigneten Wirt treten diese Veränderungen nur selten auf, und der Parasit kann sich nicht entwickeln. Verschiedene Autoren haben vermutet, dass die resistente Wurzel eine chemische Substanz enthält die dem Nematodenspeichel entgegenwirkt.

Ein solche Substanz könnte auch auf andere Weise die Pflanze schützen, z.B. durch Verdeckung der Sekretion die die Nematoden anzieht oder durch einfache Giftigkeit für die Nematoden

Um ausfindig zu machen wie die natürliche Resistenz wirkt, und um experimentell zu versuchen einen Zustand von Resistenz in einer empfindlichen Pflanze oder Pflanzenteil hervorzurufen, werden neue Methoden benötigt. Diese Arbeit beschreibt die Entwicklung solcher Methoden und berichtet über ein vorbereitendes Experiment, das hilft den örtlichen Ursprung der Resistenz klarzustellen. Isolierte Wurzelkulturen von *Lycopersicon peruvianum* blieben gegen *Meloidogyne incognita* nach 27 Transplantationen in Nährlösung resistent. Dies zeigt dass die Resistenz von dem wachsenden Wurzelgewebe ständig erneuert wurde, und nicht von der Übertragung von Stoffwechselprodukten aus dem überirdischen Teil der Pflanze abhängig war.

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SCUTELLONEMA CLATHRICAUDATUM N. SP. (HOPLOLAIMINAE: TYLENCHIDA), A SUSPECTED ECTOPARASITE OF THE ROOTS OF THE COTTON PLANT (*GOSSYPIUM HIRSUTUM* L. VAR. UK 51)

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Two samples of soil from around the roots of cotton, *Gossypium hirsutum* L. var. UK 51, collected from a site in Tanganyika which had been planted to cotton for fifteen consecutive years, contained 5000 and 1000 specimens respectively, per litre of soil, of a new species of *Scutellonema* Andrássy 1958. This species is believed to be an ectoparasite of cotton roots but its pathogenicity is unknown.

Scutellonema clathricaudatum n. sp.

15 ♀♀: L = 0.596-0.747 mm; a = 19.1-25.4; b = 4.7-6.9; c = 27.0-47.5; V = 51.2-60.3 %. Spear = 21-25 μ.

♂♂ unknown.

Holotype: L = 0.665 mm; a = 22.2; b = 5.7; c = 47.5; V = 60.0 %.

Body stout, fixed specimens take the form of the letter C.

Head shaped like truncated cone, with typically seven annules, occasionally six. Skeletal framework of head strongly developed (Fig. 1C), the extremities of basal plate extending back into body half width of first body annule.

Body cuticle coarsely annulated, annules on middle region about 2 μ wide. Lateral fields arise a few annules behind the head. Each field has four somewhat crenate incisures for greater part of its length. There are traces of striation on the two outer bands but cross striation of the central band was only observed towards the anterior end of the body and on the tail. Beyond the large scutellum, which overlaps the central incisures, the lateral field is cross striated, though the striations

of the central band may be oblique (Fig. 1F). Cross striation also extends for two or three annules anterior to the scutellum. Striation of the tail follows the tail contour but in those specimens with an indented terminus the striations are transverse. As the amount of in-

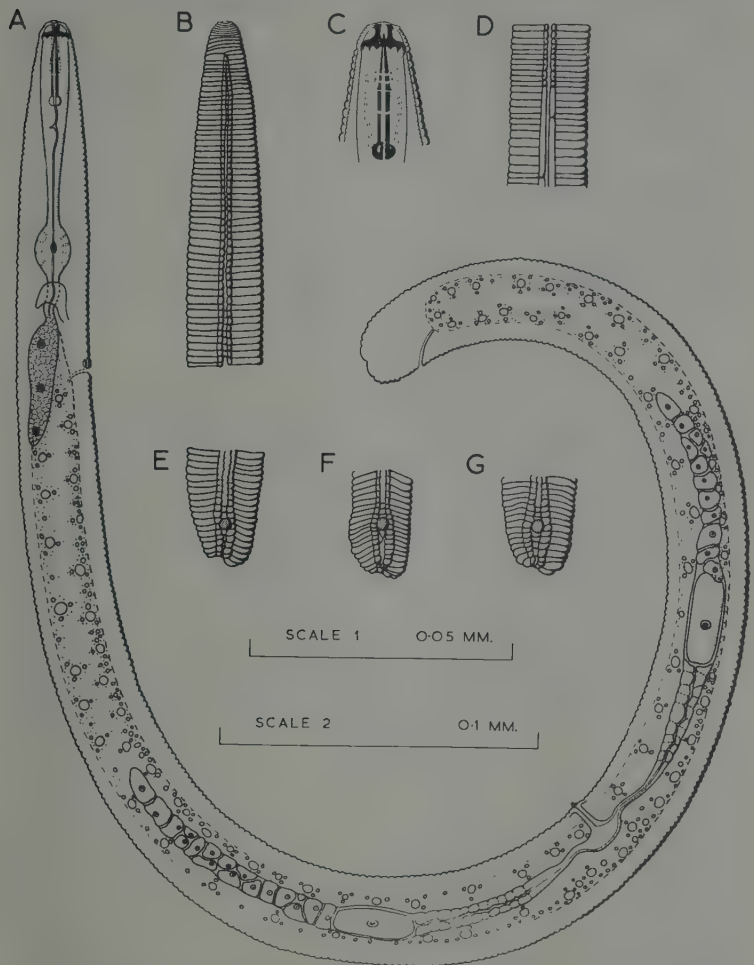

Fig. 1. A, *Scutellonema clathricaudatum* ♀. — B, Anterior part of the lateral field. — C, Enlarged lateral view of head and anterior body region. — D, Lateral field immediately behind the excretory pore. — E, F, G, Cuticular patterns of the tails of three specimens.
A, B & D-G are drawn according to scale 2. — C is drawn according to scale 1.

dentation varies considerably it is not felt that types with indented tails represent a separate race.

Spear around 23 μ long and carries more or less rounded basal knobs on which fine indentations of the anterior margins were observed in several specimens. The spear guiding apparatus appears as three rings around the spear (Fig. 1C).

Oesophageal gland cells contained in a single lobe overlapping intestine dorsally. Dorsal oesophageal gland duct opening into oesophageal lumen about a quarter of a spear's length behind spear base.

Intestine highly granular, obscuring details of reproductive system in about half the specimens examined. Rectum and anus however, well developed.

Excretory pore difficult to observe, situated between anterior and posterior extremities of glandular region of the oesophagus. Some thickening of cuticle immediately anterior to excretory pore in most specimens; this may constitute the hemizonid.

Ovaries paired, oocytes being arranged in tandem, except a short region towards free ends; here two rows observed. Uterine region of reproductive system extremely difficult to see clearly. Spermathecae absent, and this would appear to be correlated with the absence of males. Vulva situated a little behind middle of body.

Type specimens: Holotype ♀ No. 2 slide G.h. 13 in slide collection of Nematology department, East African Agriculture and Forestry Research Organisation, P.O. Box 21, Kikuyu, Kenya.

Type locality: around the roots of *Gossypium hirsutum* L. var. UK 51, in fifteen-year-old cotton monoculture, Empire Cotton Growing Research Station, Ukiriguru, P.O. Fela, Lake Province, Tanganyika.

DISCUSSION

In a recent paper (ANDRÁSSY, 1958) a revision of the subfamily *Hoplolaiminae* Filipjev 1936 was proposed. Species bearing very large phasmids, or scutella, having rounded spear knobs and the central band of lateral field smooth, were placed in a new genus *Scutellonema*. To this genus the new species described above must be assigned.

S. clathricaudatum is anatomically related to the species *S. blaberum* (Steiner, 1937) Andrássy, 1958, *S. boocki* (Lordello, 1957) Andrássy, 1958, *S. bradys* (Steiner & Le Hew, 1933) Andrássy, 1958 and *S. coheni* (J. B. Goodey, 1952) Andrássy, 1958. In all five species the lateral field in the region of the scutellum is areolated to a varying degree, as distinct from *S. brachyurum* (Steiner, 1938) Andrássy, 1958,

and *S. christei* (Golden & Taylor, 1956) Andrássy, 1958, which do not exhibit such areolation. Of the former group of five species only *S. boocki* and *S. clathricaudatum* exhibit any areolation of the central band of the lateral field. In *S. boocki* LORDELLO draws a single stria across the central band corresponding to the first body annule in front of the scutellum. In *S. clathricaudatum* however, the central band of the field is areolated between its terminus on the tail and the scutellum and for two or three annules in front of the scutellum. Moreover, *S. clathricaudatum* has typically seven head annules, whereas the head of *S. boocki* carries five annules.

Diagnosis. — *Scutellonema* with the above characters. Head with seven annules. All three bands of the lateral field fully areolated behind and for two or three annules in front of the scutellum.

Acknowledgements. — The writer is very grateful to Mr. F. G. W. Jones and Dr. J. Basil Goodey of the Nematology Department, Rothamsted Experimental Station for valued criticism of the text.

RESUMÉ

L'auteur donne une description de *Scutellonema clathricaudatum* n.sp., ecto-parasite présumé des racines du coton. Cette espèce est morphologiquement apparentée à *S. blaberum, S. boocki, S. bradys* et *S. coheni.* Ces 5 espèces possèdent un champ latéral aréole à un degré variant dans la région du scutellum et se distinguent ainsi de *S. brachyurum* et *S. christei.* Des 5 espèces précitées seules *S. boocki* et *S. clathricaudatum* possèdent une bande centrale du champ latéral qui est aréolée (notamment les bandes derrière le scutellum et les deux ou trois anneaux devant celui-ci). La tête de *S. clathricaudatum* possède 7 anneaux, celle de *S. boocki* n'en possède que 5.

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THE BEHAVIOUR OF LARVAE OF *HETERODERA SCHACHTII* ON NITROCELLULOSE MEMBRANES

BY

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Although there has been considerable study of the behaviour of fungal pathogens on host cell wall imitations, no record has been found of such a study with any nematode. To achieve passage by force through a cell wall, it has been shown that a pathogen must first be firmly anchored. In fungi, anchorage is obtained by adhesion between the wall of the pathogen and that of the host cell, and passage through the latter follows with or without the softening of the wall by enzyme action.

LINFORD (1942), describing the entry into roots of the root-knot nematode, wrote "a larva thrusts its stylet chiefly when its body is so braced that it may hold its head firmly against a resistant surface". He made no mention of how such bracing was achieved. In entry of *Schistosoma mansoni* (Cestoda), into mammalian skin described by GORDON & GRIFFITHS (1951) and GRIFFITHS (1953), the adhesion of the cercariae was obtained by the anterior suckers. GRIFFITHS (1953) wrote "the cercaria with tail attached, makes its attack upon the stratum corneum at right-angles to the skin surface, and by mechanical body and tail movement, assisted by material ejected from the anterior cephalic glands, creates a minute puncture in the stratum corneum". It would appear that suction was the means for the essential adhesion (or anchorage) in *S. mansoni,* and that penetration was achieved by a combination of enzyme action and body movement. The latter was rapid and from side to side. There was also a considerable elongation of the body.

In *H. schachtii,* the means of adhesion are unknown, but the back thrust to mechanical penetration (i.e. LINFORD's "body is so braced") might be taken up either by the surface tension of the water film, or by the inertia of the soil particles around he roots. No reference has been found in the literature to suction between the fused lips and the root surface. The object of the present study was to find out how an eelworm larva penetrates the outer cell wall of the root apex.

METHODS

The type of nitrocellulose used was Messrs. I.C.I. H.L. 30/40. 23.48. The stock solution was 0.4% in 9/91 : alcohol/ether. Paraffin wax/m.pt. 52°C was dissolved at the rate of 0.5% in ether. The parent solution for making membranes contained 2 ml of nitrocellulose, 3 ml. of 9/91 : alcohol/ether and 0.7 ml of ether, with or without wax. The two types of membranes were made on a perfectly clean level glass plate by dropping three drops of this mixture from a pipette held vertically one inch above it. Such a volume of solution spread to cover a circle of about 3 cm in diameter. After evaporation of the solvents, this had a rim of about 1 mm in width, and within it an even film, coloured blue by reflected light. The thickness and therefore the colour of the membrane could be varied by altering the amount of nitrocellulose. After evaporation the glass plate was momentarily immersed in water. Any drops of water present were shaken from the surface of the membrane which was then gently floated off on to water. At this stage selection of membranes was rigorous. Only those truly circular with a diameter of 3 cm and coloured blue were kept. Van Tieghem cells (Dickinson, 1949), were modified for testing the behaviour of larvae on such membranes. They had the membrane as a horizontal partition, thus dividing them into two compartments. Distilled water only was used in the lower compartment. The rings comprising the cells were fastened together with vaseline.

The larvae used were freshly hatched. After repeated centrifuging and decanting, they were suspended in distilled water. The angle of contact between the drop of larval suspension and a wax-free membrane was low, i.e. below 70°, therefore the membrane surface was hydrophilic. The angle of contact between the suspension and a membrane containing wax was high, i.e. about 120°, therefore this membrane surface was hydrophobic.

EXPERIMENTAL EVIDENCE

In Van Tieghem cells, the larvae wriggled along the membrane surface, and only left it under the conditions described below. Larvae moving freely in a film of water less than their own diameter have not been observed attempting to fasten themselves to the membrane. Recently Wallace (1958) has shown that a maximum speed was attained in a film of 2-5 μ in thickness. On a hydrophilic surface larvae at the edge of a liquid drop have been seen to make unsuccessful attempts both

to move out of the water drop and to extend its boundary. Larvae on a membrane in a liquid at least ten times as deep as their diameter, made frequent attempts to fasten their anterior ends to it. Less frequently the posterior end has been seen stuck or fastened to the membrane for a short period.

When the Van Tieghem cells were inverted, the larvae came quickly to the water/air interface. Stationary larvae often became active under these conditions within a few hours. They have not been observed penetrating the water/air interface.

The first indication of an attempt by a larva to fasten its head to a membrane was a lifting up of the head away from the membrane surface. The head was then turned at an acute angle towards the membrane, a movement which pushed the fused lips into contact with its surface. An attempt was then made to lift the body into a position vertical to the membrane.

On a wax-free membrane the vertical position was seldom attained, but if achieved, was rarely held for more than a few seconds. On a membrane containing wax, i.e. hydrophobic, the vertical position was often held for more than half an hour. During this time the head remained in a fixed position and the rear part of the body swung and rotated. The head always remained stationary, while the speed of rotation varied. The body did not elongate.

In the full vertical position it was only possible to observe the basal knobs of the stylet at one level of focus. These often moved out of focus probably as a result of stylet extrusion. A supposition which was confirmed when the larva swayed from the vertical position. Then it was possible to focus both part of the stylet and knobs and so to confirm stylet extrusion. Lateral movement of knobs was seen, and this, it is presumed, resulted in a variation of the direction of thrust of the stylet. Sometimes the larva swayed so far from the vertical position that it was possible to see the stylet protruding from the fused lips, and below the membrane.

Stylet extrusion varied from a few single thrusts to repeated extrusions for some minutes at a rate of about twenty to forty in 30 seconds, and sometimes continued after the adhesion had been broken. No pulsation of the esophageal bulb has been recognised, but a thread of saliva (LINFORD, 1937) has often been observed hanging from the mouth of larvae not adhering to the membrane. Occasionally what appeared to be saliva was seen below the membrane at the point to which a larva had adhered and had been seen to move away. The result of

stylet extrusion would appear to produce either a relatively large hole in the membrane, or a series of fine holes close together. So far neither has been demonstrated. Passage right through other membranes has been observed, and there is a little evidence which might suggest the presence of a pectolytic enzyme in the saliva.

On moisture proof cellophane, adhesion took place, and the vertical position was attained. However, this was kept only for a few minutes. On 1.5% agar or 15% gelatine the vertical position has never been seen.

An experiment was designed to investigate the difference in behaviour between larvae on wax-free (hydrophilic) and wax containing (hydrophobic) nitrocellulose membranes, see Table I. Fifteen membranes were prepared as described, six contained no wax, and nine had wax in the parent mixture. Three from both mixtures were kept on water for 24 hours and then mounted in Van Tieghem cells. Three from both mixtures were soaked in 90% alcohol for 24 hours, floated on to a large volume of water and then mounted. The remaining three membranes containing wax were kept on water for 24 hours; and, whilst still wet, were put for five minutes into a closed dish containing 4/1 : alcohol/ether vapour and then mounted.

Three separate drops of a larval suspension were placed on each of the fifteen membranes, and the Van Tieghem cells were then closed by coverslips. The drops varied in diameter between 2 and 5 mm and the average larval content was 7.2. Each drop was observed for a period of 60 to 90 sec. 65-70 minutes were required for the examination of all the 45 drops used. Three examinations were made in the morning and three in the afternoon of the same day. The magnification used varied between 100 and 155.

Records of their behaviour depended upon whether the larvae were vertical or attempting to attain that position during the time of scrutiny. An arbitrary time limit had to be fixed to distinguish between those which failed and those which succeeded in retaining the vertical position. Previous observation had indicated that on wax-free membranes the vertical position was never retained for more than 20 sec. The criteria adopted then was retention of this position for less than 20 sec, and its retention for the whole period during which the particular larva was under scrutiny, i.e. between 60 and 90 sec.

Statistical analysis of the results showed that the number of larvae in the drops did not fall into a Poisson distribution, probably because

of settling in the vertically held pipette during the process of putting the drops on the membranes. There was no significant difference between the records from the different alcohol treatments. There were, however, significant differences between the records of behaviour on the wax-free and the wax containing membranes.

This analysis was based on the ratios of larvae attaining the vertical position for 20 sec or less to those retaining the vertical position for 60-90 sec on the two types of membrane.

The table shows the totals for each form of behaviour at each examination.

TABLE I

Experiment demonstrating the difference between the behaviour of larvae of Heterodera schachtii *on wax-free and wax containing nitrocellulose membranes.*

Membranes contained: —		No wax		Wax	
Number of membranes used: —		6		9	
Total number of larvae: —		120		204	
Number of larvae retaining vertical positions for: —		20 sec or less	60-90 sec	20 sec or less	60-90 sec
1st scrutiny		6	0	2	15
2nd „		6	0	1	18
3rd „		10	0	2	14
4th „		19	0	7	21
5th „		13	1	4	22
6th „		13	0	3	22
	Total	67	1	19	112

The ratios 67/1 : 19/112 were shown by a χ^2 test to be significantly different at the 0.1% level.

In addition significant differences between the numbers of attempted adhesions were found between the three morning and the three afternoon examinations. This may well have been due to the rise in temperature during the day.

DISCUSSION

The hypothesis is put forward that the essential anchorage between larva and host root (that is LINFORD's "its body is so braced") is achieved by suction developed between the fused lips and the surface to be penetrated. This suction can only be built up when the fused lips adhere to a suitable surface. Hydrophobic nitro-cellulose is such a surface; hydrophilic nitrocellulose is not.

It is suggested that successful adhesion is achieved because a water film cannot be easily interspersed between the fused lips and the surface of the wax containing membrane. On a wax-free membrane, adhesion can be achieved, but as soon as suction starts to develop water would be drawn through the hydrophilic nitrocellulose. This water would break any adhesion previously achieved and so prevent suction being developed. It is thought that suction is necessary for satisfactory adhesion in order first that the body may be hoisted into a vertical position, and second to take up, when the stylet is extruded, the inevitable back thrust of the membrane.

The stimulus for adhesion can only be a physical one. Any hydrophobic surface would appear suitable. No stylet extrusion was observed on moisture-proof cellophane, which is very much thicker than the nitro-cellulose membranes. Otherwise stylet extrusion appeared to follow adhesion — perhaps only successful adhesion — and if so would also be a reaction to a purely physical stimulus. From such a conclusion, it might be suggested that root hairs were not invaded because their walls were made of cellulose and calcium pectate (CORMACK, 1949) and therefore hydrophilic. There is also the possibility that the convexity of their surface would prevent successful adhesion, in other words the ratio between the diameters of the root hairs and the fused lips of the larva must be below a certain value before adhesion can take place. Adhesion could take place on the surface of the cell walls on the outside of the root provided it was hydrophobic, a condition achieved as soon as suberisation of the walls began.

Adhesion and attempted penetration being a response to a purely physical stimulus it could take place on any hydrophobic root surface. In other words specificity of host root would only have an effect after penetration of the cell wall by the stylet. It is probable that any attractive or repellent effect of root diffusates, if present, would exert their influence before the root surface was reached.

This conclusion is in agreement with the view that the range of entry into host tissues is considerably wider than the range of recorded host plants (A. M. SHEPHERD *in litt.*).

I am grateful to Miss S. Vincent for the statistical analysis, and to Dr. F. Moriarty, for the larval suspensions. Thanks are given to Messrs. Imperial Chemical Industry, Ltd. for the provision of nitrocellulose.

ZUSAMMENFASSUNG

Das Verhalten der Larven von Heterodera schachtii *an Nitrozellulose-Membranen*

Larven von *Heterodera schachtii* können sich anheften und ihren Stachel durch wachshaltige Cellulose-Membranen stossen. Das Anhaften an wachsfreien Membranen dauert selten länger als wenige Sekunden. Es wird angenommen, dass das Anhaften und der Versuch, in die Wurzeloberfläche einzudringen, auf einem rein physikalischen Reiz beruht. Spezifität für besondere Wirtswurzeln zeigt sich erst, nachdem das Einstossen des Stachels beendet ist.

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A RAPID METHOD FOR THE TRANSFER OF NEMATODES FROM FIXATIVE TO ANHYDROUS GLYCERIN

BY

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The existing rapid methods for making permanent mounts of nematodes (such as the lactophenol technique of FRANKLIN & GOODEY 1949) do not always give lasting results or require much manipulation of the nematodes as in the two methods by BAKER 1953. The slow method for transferring nematodes to anhydrous glycerin (see GOODEY 1957) is excellent but the experience required to obtain the right speed of evaporation and the long time which elapses between collecting the nematodes and having them ready for mounting has kept several nematologists from using it.

The slow penetration of the glycerin into the nematode tissue in this method appeared not to be due entirely to the properties of the glycerin but largely to the use of water as a solvent. When the glycerin was dissolved in methanol it penetrated much more rapidly and ethanol was still better in this respect.

Well fixed, nematodes can be transferred to pure methanol without suffering any shrinkage provided the methanol is not colder than 40°C. If the methanol contains some glycerin the solution can be concentrated to pure glycerin in a period varying from about three hours at 50°C to twenty minutes at 64°C (the boiling point of methanol) without causing shrinkage. At room temperature concentration must be slowed down to a period varying from several days to two weeks.

A solution of five parts of glycerin in 95 parts of 96% ethanol can be concentrated to pure glycerin at 30°-40°C in three hours without shrinkage of the nematodes it contains.

However, fixed nematodes are severely damaged when transferred to cold or hot 96% ethanol. The highest safe concentration is 30%. From thereon it must be increased gradually to 96% ethanol. If this is

done in not less than twelve hours at 30-40°C nematodes will not shrink.

The nematodes are transferred from the fixative to a small quantity of 20% ethanol in a small syracuse watch glass, or other suitable container, which is then placed in a closed glass vessel or desiccator containing an excess of 96% ethanol. The 20% ethanol attracts ethanol from the saturated atmosphere and the water evaporates and is attracted by the 96% ethanol in the vessel. It is advisable to add some glycerin (about 1%) to the 20% ethanol. Sometimes, probably due to small differences in temperature in the vessel, the syracuse watch glass runs dry if it contains only ethanol and water. Glycerin cannot evaporate rapidly and attracts ethanol.

Although the transfer from fixative to hot methanol does not cause shrinkage of the nematodes, their internal structure sometimes becomes too grainy. A gradual increase of the methanol concentration gives excellent results but then the time and work involved are the same as when ethanol is used. The latter is to be preferred over methanol as it allows the concentrating of the glycerin to be done at a lower temperature.

FIXATION

The final condition of the internal structures of the nematodes processed by the procedures described above depends on the method of fixation. Both F.A. 4 : 10 and F.A.A. appeared to be good fixatives. The results with T.A.F. (Courtney, Polley & Miller 1956) were less satisfactory. It seems that this alkaline fixative does not harden the tissues sufficiently. With a mixture of TAF (4 parts) and F.A. 4 : 10 (1 part) having a pH of about 3 the results are very good.

Procedure for processing nematodes through 96% ethanol to glycerin.

The procedure recommended above can now be summarized as follows:

1. Transfer nematodes from fixative to a syracuse watchglass or other suitable container with 0.5 ml of a mixture of:

96% ethanol:	20 parts
glycerin:	1 part
dist. water:	79 parts

2. Place container with nematodes in closed glass vessel or desiccator

containing an excess of 96% ethanol and keep at 35-40°C for at least 12 hours.

3. After this, fill container with a solution of:

   5 parts glycerin in 95 parts of 96% ethanol.

4. Place container in a partly closed petridish and keep at 40°C until all ethanol has evaporated. This should take at least 3 hours.

5. Place container in desiccator with dry silicagel or calcium chloride, or mount nematodes in anhydrous glycerin immediately.

If shrinkage should occur in stage 2, add water and concentrate slowly to 96% ethanol again. If shrinkage occurs in stage 4, transfer nematodes to fresh ethanol-glycerin mixture, and repeat stage 4.

ZUSAMMENFASSUNG

Schnellmethode zur Überführung von Nematoden aus der Fixierungsflüssigkeit in wasserfreies Glyzerin

Eine Methode wird beschrieben bei der Nematoden innerhalb 24 Stunden von der Fixierungsflüssigkeit in wasserfreies Glyzerin überführt werden. Die Nematoden werden nach Fixierung in ein Gemisch von 20 Teilen Aethanol 96%, 1 Teil Glyzerin und 79 Teilen Wasser gebracht. Das Aethanol in diesem Gemisch wird durch Wasserentzug im Exsiccator mit Aethanol 96% bei 35°-40°C in nicht weniger als 12 Stunden bis zu etwa 95% konzentriert. Danach wird ein Gemisch von 5 Teilen Glyzerin in 95 Teilen Aethanol 96% hinzugefügt. Dieser wird bei 40°C in wenigstens 3 Stunden zu reines Glyzerin konzentriert.

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NOTHANGUINA CECIDOPLASTES N. COMB. SYN. *ANGUINA CECIDOPLASTES* (GOODEY, 1934) FILIPJEV 1936 (NOTHOTYLENCHINAE: TYLENCHIDA)

BY

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VENKATARAYAN (1932) described galls on the leaves, stems and flowers of *Bothriochloa pertusa* (Willd.) A. Camus, syn. *Andropogon pertusus* Willd. GOODEY (1934) described the nematodes inhabiting these galls and assigned them to a new species of the genus *Anguillulina* Gervais & van Beneden, 1859. Subsequently the genus *Anguillulina* was split by FILIPJEV (1936) into a number of clearly distinct genera and *A. cecidoplastes* was placed in the genus *Anguina* Scopoli, 1777.

In reviewing the descriptions of *Anguina* spp., the author noticed that *A. cecidoplastes* differed from all other *Anguina* spp., in the absence of a median oesophageal bulb. FILIPJEV & SCHUURMANS STEKHOVEN (1941) also pointed out that this character was shared with *Iotonchium* Cobb 1920, although in other respects the two genera were quite distinct. THORNE (1949) erected a new family *Neotylenchidae* to receive those genera, lacking a median oesophageal bulb. The author now proposes that the nematode *Anguina cecidoplastes* (Goodey, 1934) Filipjev 1936 be placed in this family, and erects a new genus — *Nothanguina* (sub-family *Nothotylenchinae* Thorne, 1941) to receive it. This new name indicates the relationships of the nematode, i.e. on strict morphological grounds with Nothotylenchinae, though retaining many features of secondary taxonomic importance shared with *Anguina* spp.

Nothanguina n.g.

Order: Tylenchida Thorne, 1949;
sub-family Nothotylenchinae Thorne, 1941.

Diagnosis. — Body stout, ventrally coiled in female; head flattened, with six lips; small knobbed spear; oesophagus without median bulb,

narrowing to slender isthmus behind aperture of subventral oesophageal glands; gonad single, prodelphic, reflexed anteriorly; no distinct spermatheca in female but with a post-vulval uterine sac. The male has a ventral bursa not extending to the tail tip, but no gubernaculum. The tails of both sexes taper to a minute digitate, terminal process.

Type species: *Nothanguina cecidoplastes* (Goodey, 1934) n. comb. syn. *Anguina cecidoplastes* (Goodey, 1934) Filipjev, 1936.
Anguillulina cecidoplastes, Goodey, 1934.

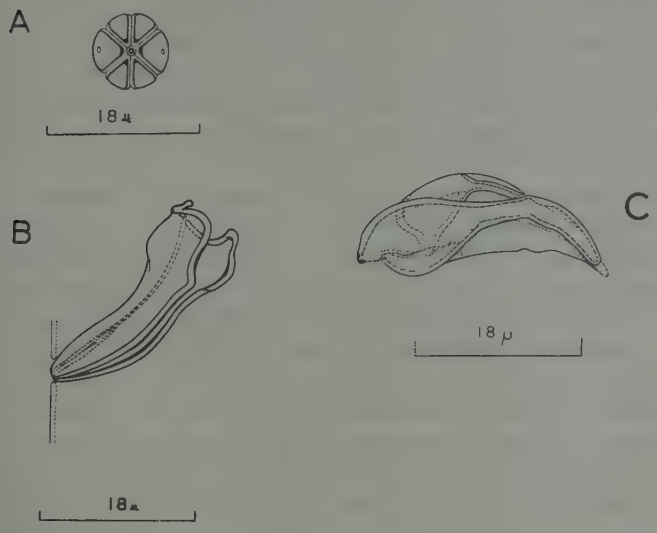

Fig. 1. *Nothanguina cecidoplastes*. (The drawings of Goodey, 1934 are adopted as illustrations of the type species with the substitution of B and the addition of A & C.) A ♀ head, *en face* view; B lateral view of spicule; C dorso-lateral view of spicules.

Measurements. — ♀♀ L = 1.2-2.03 mm, a = 12.8-18; b = 9-13; c = 18-31.5; V = 88-93%; stylet = 4-9 μ (After Goodey, 1934). ♂♂ (9 specimens) L = 0.94-1.33 mm, a = 16.2-23.2; b = 6.6-8.0; c = 12.6-17.6; spicules = 21-28 μ.

The measurements of female worms agreed very closely with those of Goodey (1934) and apart from the stylet measurement are quoted unchanged. Several specimens were however found to possess a very small spear of about 4 μ length. Goodey (1934) illustrates a spear 6.9 μ long, but gives the spear measurements as 8 — 9 μ. The measure-

ments of the male agree with those of GOODEY but the spicule length was found to be rather less, 21 — 28 μ. GOODEY's measurements gave 28 — 30 μ, moreover he gave c as 12.4 — 14.

Morphology. — Head flattened, inverted saucer-like in shape, about 9 μ wide and approximately six times as wide as long. Margins of head somewhat angular, a slight constriction separating it from rest of body. Mouth situated in slight depression on anterior face of head, which has six lips, subdorsal and subventral pairs being of equal width, while lateral lips somewhat wider. Amphidial pore situated towards outer edge of lateral lip. No striations of lateral head margins observed.

Buccal cavity bears small spear, 4 — 9 μ long of usual tylenchoid shape, with three small, rounded basal knobs. Closely adherent to pointed anterior end of spear are the cheilorhabdions, 0.5 μ long, forming a spear guide.

Oesophagus divisible into three sections, viz. an anterior non-muscular corpus, somewhat swollen in region of aperture of sub-ventral oesophageal glands but without true median bulb, an isthmus, and a posterior glandular region composed of three gland cells, two small subventral and one larger dorsal cell. Each gland cell has large nucleus. Dorsal oesophageal gland duct aperture lies behind base of spear a distance up to 14 μ.

Intestine a simple, thick-walled tube, connecting anteriorly with oesophagus without valvular apparatus, and posteriorly with thin walled, narrow rectum about 25 μ long leading to anus.

Excretory pore situated midway opposite glandular portion of oesophagus.

Female. Body very stout, tapering in front from oesophago-intestinal junction to head and behind from posteriorly placed vulva.

Single ovary, extending to vicinity of oesophago-intestinal junction, exhibiting two flextures, first lying ventrally or dorsally to second, which may reach as far forward as oesophago-intestinal junction. In cross section oogonia and oocytes appear to have a radial disposition about a rachis. Posterior region of ovary occupying considerable volume of body and ending in sphincter of large cells, separating it from anterior part of uterus or receptaculum seminis composed of flat, parenchyma-type cells. Receptaculum seminis short ending in another large-celled sphincter, behind which lies uterus. Uterine wall thin for three quarters of its length, prevulval quarter thick walled. Latter region,

together with postvulval uterine sac, probably assisting in expulsion of ova through vagina.

Male. Body stout and fusiform. Gonad has single flexure, lying ventrally, not extending to oesophago-intestinal junction. In posterior third gonad forms thick-walled vas deferens, passing ventrally as narrow ejaculatory duct, between spicules, to cloaca.

Spicules rather complex in structure, bursa ventral. Bursal wings arising low on subventral surface of body, just in front of heads of spicules, and extend to point halfway between cloaca and tail tip. No trace of gubernaculum.

Spicule has heavy appearance and point is blunt. In cross section these points have quadrate form; two dorsal thickenings of points correspond to dorsal strengthening ridges of shaft and head, ventral thickenings of points extend some distance into floor of shaft but unlike dorsal thickenings do not extend into head. Dorsal ridges separated by suture, which extends forward to head of spicule, opening out on head which is, in consequence, open both dorsally and proximally. Ventral suture runs along floor of spicule head and some way into shaft. Dorsal ridges come close together at proximal end of spicule head and give appearance of ventral digitate process. Each spicule appears to be composed of two halves separated wholly by dorsal and partly by ventral sutures. GOODEY's drawing of spicule rather misleading as head is drawn as 'closed' structure, while dorsal ridges are said to be ventral, due to drawing of dorsal surface of spicule in lateral position. Membraneous expansion, said by GOODEY to pass on ventral surface between head and point of each spicule, thought to be side wall of spicule, when viewed dorsally.

Eggs. GOODEY states eggs measure 85 μ to 100 μ by 31 μ to 38 μ wide. Length and breadth measurements of egg last in uterus found to be 84 — 93 μ x 34 — 37 μ which agrees well with GOODEY's figures. The eggs are laid segmented.

Larvae. According to GOODEY there are only two larval stages, first measuring 0.53 mm to 0.66 mm and freshly killed second stage larvae measuring 0.737 mm to 0.805 mm. However, in his file of original measurements size of infective second stage larvae ranges from 0.6 to 0.66 mm, when recovered from fixed galls. This gives a contraction figure for fixation of 18%. Measurements made of many larvae ex-

tracted from fixed galls when increased by 18% give measurements agreeing closely with those of GOODEY but a few larvae measuring 0.3 to 0.33 mm, which do not fit in with this range for GOODEY's first stage larvae, also found. These larvae were undergoing a moult and were very close in size to larvae in ecdysis found within the egg, which suggests that the first moult occurs within the egg. Thus the larvae hatching from the egg are believed to be second stage larvae which in turn moult to form third stage, infective larvae and which after penetration of host tissue give rise after another moult to young adult worms. Clear proof of this is, however, impossible, until further fresh galled grass specimens are available.

First stage, fixed, larvae, therefore measure 0.3 to 0.33 mm. No rectum or anus. Gonad very simple, consisting of one large cell and perhaps two very small cap cells, situated ventrally to intestine about halfway along length of intestine.

Second stage larvae measure 0.53 to 0.66 mm in length according to GOODEY and possess stylet, oesophagus, intestine and undeveloped gonad. These larvae moult to give third stage larvae.

Third stage or infective larvae, according to GOODEY, measure 0.737 to 0.805 mm. Head offset by slight constriction and has truncated cone shape. There are six lips. Lateral fields occur at sides of body and each field is marked by four incisures and is approximately one eighth as wide as body. Stylet measures 6 — 7 μ. Oesophagus has same structure as in adult and agrees with it in absence of median bulb. Tail bears terminal process as does the adult. Gonad enlarges longitudinally just prior to ecdysis.

Developing adults have stout, fusiform body. Head offset and retains at first shape of truncated cone. Gonad well developed, cigar-shaped while in some specimens flexure apparent. Gonad made up of rows of oogonia. Vulva present in young female worms with well developed ovaries, while head becomes flattened disc.

DISCUSSION

Nothanguina cecidoplastes bears a number of superficial similarities with *Anguina* spp. and these have been reason for placing this nematode in latter genus. Like *Anguina* spp. body stout and ventrally coiled in the female, head flat with six lips, oocytes arranged about a rachis, while gonad is reflexed; there is post-vulval uterine sac. Striae are fine and spicule blades wide. However, certain of these characters would be expected of nematodes inhabiting galls. Thus, the production of large

numbers of larvae, necessary in gall-forming nematodes of shoot structures in view of the unfavourable conditions likely to obtain on larval emergence, requires larger gonad in the female and flexure of gonad together with its larger size require that body be stout and, in consequence, the head assumes a flattened disc shape.

While gall-forming habit may explain many of the superficial similarities between *Nothanguina* and *Anguina*, there remain a number of differences between the two genera, which separate them. The absence of a median oesophageal bulb with crescentic valves in both larvae and adults places the genus *Nothanguina* in the family *Neotylenchidae* Thorne, 1949, while the presence of six lips, the structure of the oesophagus and the presence of a post-vulval uterine sac places the genus in the subfamily Nothotylenchinae Thorne, 1941. The genus *Nothanguina* differs from other nematodes of the Nothotylenchinae in its gall-forming habit and general body shape in the absence of a gubernaculum and in the presence of heavy spicules in the male.

Acknowledgements. Grateful thanks are accorded to Dr. J. Basil Goodey for permission to study his slides and fixed grass specimens, during a period of study spent in the Nematology Department, Rothamsted Experimental Station on a Colonial Research Fellowship.

RESUMÉ

L'auteur donne une description de *Nothanguina cecidoplastes* n. comb. (synonyme *Anguina cecidoplastes* (Goodey, 1934) Filipjev, 1946). L'espèce diffère des *Anguina* spp. par l'absence d'un bulbe médian oesophagien, l'absence du gubernaculum chez le mâle, et par la confluence de la bourse à mi-chemin entre le cloaque et le bout de la queue.

Cette espèce est placée dans la sous-famille des Nothotylenchinae Thorne, 1941.

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CYLINDROCORPUS SUBG. NOV. UND *PROTOCYLINDROCORPUS* SUBG. NOV., ZWEI UNTERGATTUNGEN DER GATTUNG *CYLINDROCORPUS* GOODEY 1939

VON

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Die *Cylindrocorpus*-Arten sind eine in ihrer Morphologie einheitlich gestaltete Nematodengruppe. Lippen, Bau und Form der Mundhöhle und des Ösophagus zeigen zwischen den Arten keine wesentlichen Unterschiede. Soweit aus den Abbildungen der verschiedenen Autoren hervorgeht, finden wir bei sämtlichen Arten eine anisotope Mundhöhle, deren Metastom zudem noch anisomorph ist, wie dies bereits für die Arten der Gattung *Goodeyus* Chitwood 1933 festgestellt werden konnte (vgl. RÜHM 1956). In sämtlichen Fällen sind die Übergangsstellen, die bei zahlreichen Diplogasteriden zahnartige Differenzierungen oder Zähne besitzen, klein. Typische Zahn- und Warzenbildungen wurden bei den *Cylindrocorpus*-Arten nicht nachgewiesen. Nur bei *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. ist auf der dorsalen Übergangsstelle eine grössere, warzenförmige, cuticulare Verstärkung zu erkennen.

Arttrennende morphologische Merkmale finden wir im Bau des Weibchenschwanzes, dessen Länge und Form sehr verschieden sein kann, was im übrigen auch für den Männchenschwanz gilt. In der Gattungsdiagnose von GOODEY (1939) werden 9-10 Schwanzpapillenpaare angegeben. Der Fund des *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. mit ebenfalls 10 Schwanzpapillenpaaren wie sie bei *C.* (*Cylindrocorpus*) *longistoma* (Stefanski 1922) Goodey 1939 und *C.* (*Cylindrocorpus*) *curzii* (Goodey 1935) Goodey 1939 nachgewiesen wurden, bestärkt mich in der Auffassung, dass auch *C.* (*Cylindrocorpus*) *macrolaima* (Schneider 1866) Chitwood 1933 10 Schwanzpapillenpaare zukommen. SCHNEIDER (1866) hat wohl das 6. Paar, das sich kurz vor der sog. Dreier-Gruppe am Schwanzende befindet, übersehen. Bei allen Arten ist gerade dieses Paar entweder schwach ausgebildet oder in seiner Form gegenüber den anderen Papillenpaaren modifiziert.

Die Bursa ist nur schwach entwickelt oder fehlt nahezu völlig. Je kürzer die Schwanzspitze ausgebildet ist, desto stärker tritt die Bursa hervor. Eine pelodere Bursa ist bekanntlich innerhalb der Cylindrocorporinae (Goodey 1939) Rühm 1956 bei den *Goodeyus*-Arten vorhanden.

Der Fund des *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. (s.u.), der im weiblichen Geschlecht eine unpaare Gonade besitzt, macht die Unterteilung der Gattung *Cylindrocorpus* Goodey 1939 in zwei neue Untergattungen notwendig (s.u.). Beide Untergattungen unterscheiden sich nur im Bau der weiblichen Gonade. Allerdings handelt es sich hier um ein wesentliches Merkmal, das zumindest Untergattungen voneinander trennt (vgl. Rühm 1956). Man kann vermuten, dass die Cylindrocorporinae von einer Form mit unpaarer Gonade und wohl auch hinterständiger Vulva abzuleiten sind (vgl. *Goodeyus* Chitwood 1933). Entsprechendes konnte schon für andere Gattungen der Diplogasteriden festgestellt werden.

Die *Cylindrocorpus*-Arten sind Saprobionten, die nach den bisherigen Fundmeldungen nicht allgemein verbreitet sind. Sie fanden sich fast ausnahmslos in Substraten stärkerer Konzentration. Offenbar sind sie zur Adaption an osmotisch besonders wirksame Medien befähigt. Insekten konnten als Überträger bisher nicht ermittelt werden. Wink- und Dauerlarven wurden nicht beschrieben. Auch in der Zucht gelang es mir nicht, z.B. bei *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. Dauer- und Winklarven zu erzeugen.

Familie: Diplogasteridae Steiner 1919
Unterfamilie: Cylindrocorporinae (Goodey 1939) Rühm 1956
Gattung: *Cylindrocorpus* Goodey 1939
syn. *Cylindrogaster* Goodey 1927
Genotype: *Cylindrocorpus* (*Cylindrocorpus*) *longistoma* (Stefanski 1922) Goodey 1939

Cylindrocorpus subg. nov.

Mit den notwendigen Abänderungen übernehme ich die von Goodey (1939) in der Gattungsdiagnose zusammengefassten morphologischen Merkmale auch für die Untergattungsdiagnose:
G: Kopf mit 6 rundlichen, geschlossenen, unscheinbaren Lippen. Subventrale Lippen mit 3 Papillen, Laterallippen nur mit einer Papille und den Seitenorganen. Cheilorhabdien vorhanden. Mundhöhle lang und schmal, anisotop. Metastom anisomorph. Praecorpus des Ösophagus

kurz, deutlich zylindrisch, mit Fibrillen. Metacorpus mit schmalem Isthmus und kleiner bulbusartiger Endanschwellung; mehr zellulär. Klappenapparat fehlt. W: Vulva annähernd mittelständig, zuweilen vorderständig, Gonade stets paarig. M: Schwanz mit mehr oder minder langer Schwanzspitze. Mit meist schwach entwickelter, leptoderer Bursa. 10 Schwanzpapillenpaare vorhanden, davon stets 3 Paare präanal, 1 postanales Paar stets subdorsal. Spicula paarig, nicht verwachsen. Gubernaculum vorhanden.

In diese neue Untergattung gehören neben der Genotype: *Cylindrocorpus* (*Cylindrocorpus*) *curzii* (Goodey 1935) Goodey 1939 und *Cylindrocorpus* (*Cylindrocorpus*) *macrolaima* (Schneider 1866) Goodey 1939.

Protocylindrocorpus subg. nov.

Species typica: *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp.

Für diese neue Untergattung gilt dieselbe Diagnose wie für die Ug. *Cylindrocorpus* subg. nov. jedoch ist für das weibliche Geschlecht: Gonade stets unpaar, Vulva etwas hinterständig, mit postvulvarem Uterussack einzusetzen. Derselbe Zusatz muss auch der Gattungsdiagnose eingefügt werden.

Cylindrocorpus (*Protocylindrocorpus*) *goodeyi* n. sp.

(Abb. 1, a-c; Abb. 2, a-d)

Diese Art widme ich Herrn Dr. J. B. Goodey (Rothamsted Experimental Station, England).

W:		M:	
L:	1,150— 1,515 mm	L:	0,943— 1,515 „
D:	0,049— 0,072 „	D:	0,039— 0,056 „
Mu:	0,030— 0,042 „	Mu:	0,026— 0,030 „
Ös:	0,125— 0,152 „	Ös:	0,132— 0,170 „
V-A:	0,418— 0,505 „	Sp:	0,072— 0,600 „
Schw:	0,069— 0,095 „	Gu:	0,015— 0,020 „
a:	21,0 —23,4	Schw:	0,053— 0,060 „
b:	9,2 —10,0	a:	24,2 —27,1
c:	15,9 —16,7	b:	7,1 — 8,9
V%:	57,7 —60,4	c:	17,8 —25,3

Morphologie: G: Die geschlossenen Lippen, die Mundhöhle und der Ösophagus zeigen keine wesentlichen Unterschiede in ihrer Form und Differenzierung gegenüber der Genotype. Die Mundhöhle ist schwach anisotop. Das Metastom ist anisomorph. Die kleinen Übergangsstellen

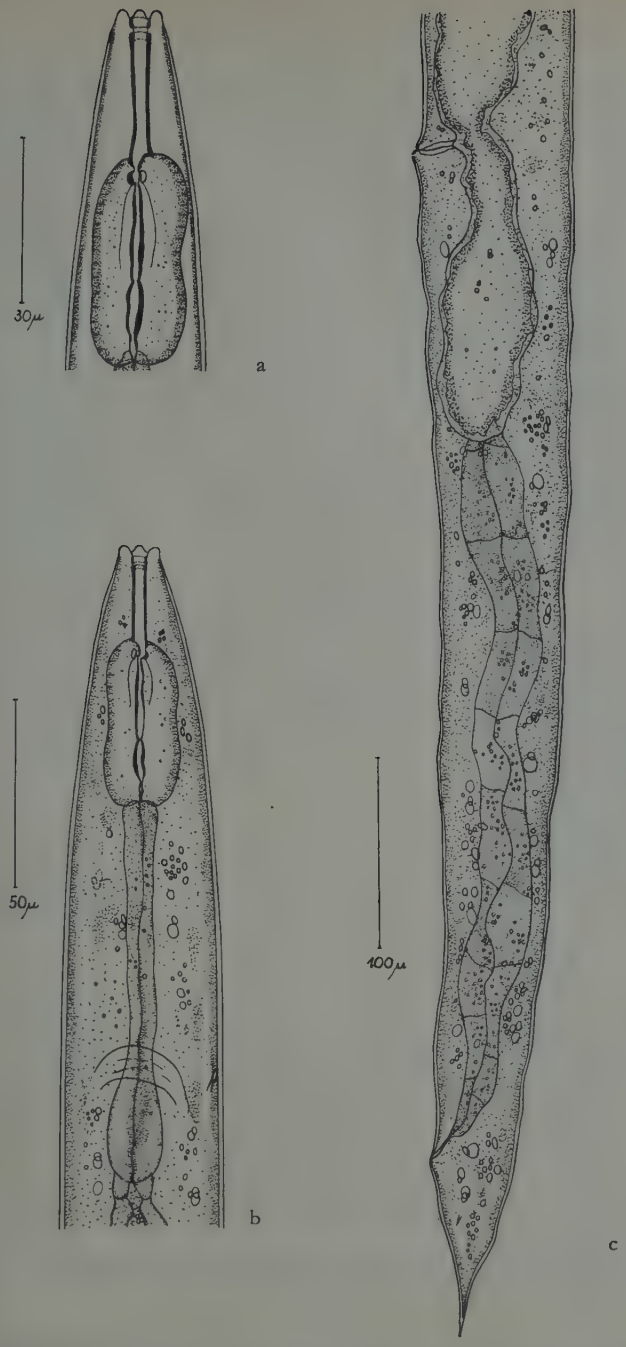

Abb. 1. *Cylindrocorpus (Protocylindrocorpus) goodeyi* n. sp. — Weibchen a.) Kopf mit Praecorpus, lateral; b.) Kopf mit Praecorpus, ventral; c.) Hinterende, lateral.

tragen keine Zahnbildungen. Dorsal ist lediglich eine grössere, warzenförmige, cuticulare Verstärkung zu erkennen (s.o.). Die Cuticula ist fein strukturiert. W: Die Gonade ist unpaar. Der lange Gonadenumschlag kann bis in die Nähe des Enddarmes reichen. Ein postvulvarer Uterussack ist deutlich ausgeprägt. Die Vulvalippen sind etwas hervorgewölbt. Der Vulvaspalt ist relativ klein. Die Vagina ist mässig cuticularisiert. Der Abschnitt V-A verjüngt sich nur langsam. Der Weibchenschwanz ist keilspitz. Er verjüngt sich rasch. M: Der Männchenschwanz trägt eine schlanke, kräftige, etwas abgesetzte Schwanzspitze. Die leptodere Bursa ist schwach entwickelt. Sie ist nicht strukturiert. 10 Schwanzpapillenpaare sind ausgebildet, von denen das 6. Paar in seiner Form stärker modifiziert ist (Abb. 2, b). Die Papillen besitzen eine fibrillenartige Versteifung. Papillenstellung prä: 1-1-1; post: 1-1-(1)-3; subdorsal: 1. Das Gubernaculum ist pantoffelförmig. Seine Form ist dem der übrigen Arten ähnlich. Es ist jedoch grösser als das des *Cylindrocorpus* (*Cylindrocorpus*) *curzii* (Goodey 1935) Goodey 1939 und des *C.* (*C.*) *longistoma* (Stefanski 1922) Goodey 1939. Besonders auffällig sind die ausserordentlich langen, dünnen Spicula. Sie sind proximal etwas verdickt. Sie sind jedoch nicht geknöpft. Nach etwa 0,120 mm sind sie eingebogen und verlaufen wellig bis in Anusnähe, wo sie schliesslich nach aussen führen. Distal sind sie fadenförmig. Die Spicula brechen distal leicht ab, so dass man innerhalb einer Population auch auf Individuen mit verhältnismässig kurzen Spicula trifft (vgl. Masse).

Biologie und Ökologie: *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. befand sich in sehr grosser Anzahl unter der Rinde von *Ceiba pentandra* Gaertn., dem sog. ‚Baumwollbaum'. Insektenfrass wurde nicht beobachtet. Das Substrat war schleimig. Das Geschlechterverhältnis ist ausgeglichen. Fundort: Basel (Schweiz) [1]).

BESTIMMUNGSTABELLE DER CYLINDROCORPUSARTEN:

1(3) Weibchen mit paariger Gonade: *Cylindrocorpus* subg. nov.
2(6) Weibchen mit unpaarer Gonade: *Protocylindrocorpus* subg. nov.
3(4) Weibchenschwanz schlank, sich stark verjüngend, jedoch nicht fadenförmig. Männchenschwanz mit kurzer, deutlich abgesetzter Schwanzspitze. Leptodere Bursa gegenüber den anderen Arten gut entwickelt: *Cylindrocorpus* (*Cylindrocorpus*) *curzii* (Goodey 1935) Goodey 1939.
4(5) Weibchenschwanz mit langem, fadenförmigem Schwanzende. Männchenschwanz mit langer, schlanker, nur etwas abgesetzter Schwanzspitze: *Cylindrocorpus* (*Cylindrocorpus*) *longistoma* (Stefanski 1922) Goodey 1939.

1) Für die Überlassung des Materials schulde ich Herrn H. Wichmann (München) Dank.

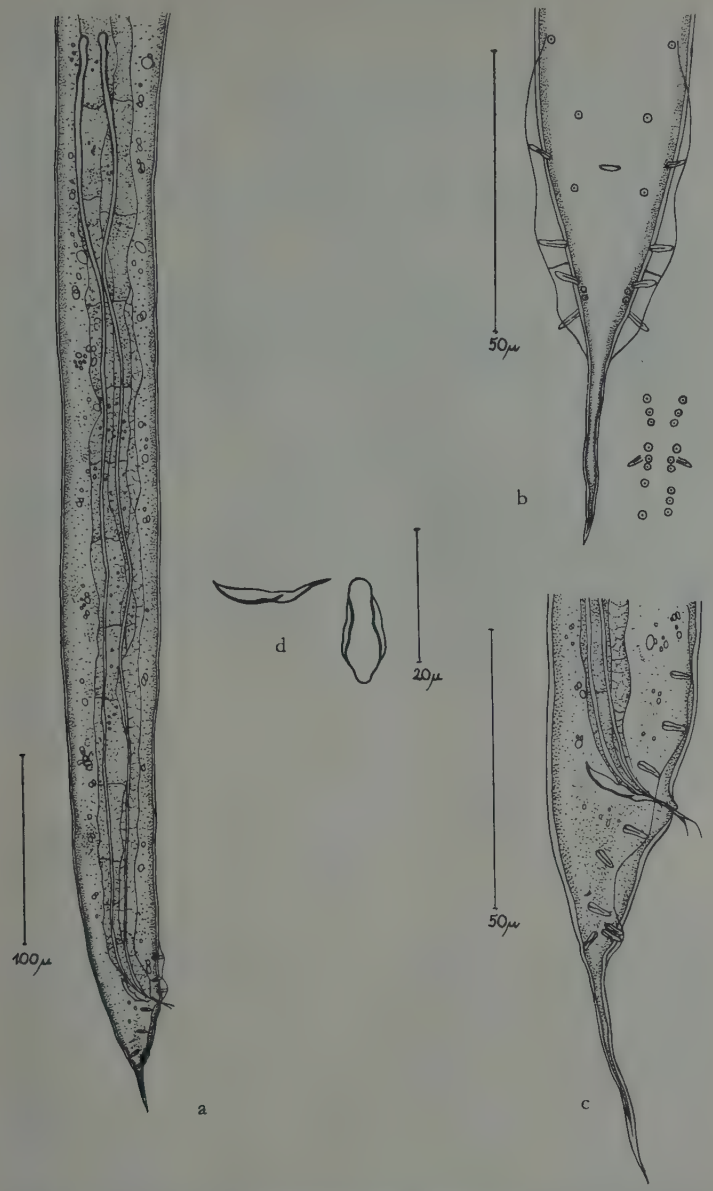

Abb. 2. *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. - Männchen. a.) Hinterende, lateral; b.) Männchenschwanz, ventral; c.) Männchenschwanz, lateral; d.) Gubernaculum, lateral und ventral.

5(—) Männchenschwanz der Genotype ähnlich, leptodere Bursa sehr schwach entwickelt. Spicula sehr dünn; sie erreichen mit ihren proximalen Enden das 1. präanale Schwanzpapillenpaar nicht: *Cylindrocorpus* (*Cylindrocorpus*) *macrolaima* (Schneider 1866) Chitwood 1933.

6(—) Weibchenschwanz keilförmig zugespitzt, sich stark verjüngend. Männchenschwanz ähnlich *C.* (*C.*) *macrolaima*. Spicula sehr dünn und aussergewöhnlich lang. Die proximalen Enden reichen weit über das 1. präanale Schwanzpapillenpaar hinaus. Die distalen Enden sind fadenförmig, z.T. sind sie abwärts gebogen: *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* **n. sp.**

SUMMARY

The genus *Cylindrocorpus* Goodey, 1939 is divided into two subgenera *Cylindrocorpus* subg. nov. with paired female gonads and *Protocylindrocorpus* subg. nov. with a single gonad. Diagnoses are made and *Cylindrocorpus* (*Protocylindrocorpus*) *goodeyi* n. sp. is described. A diagnostic key to all the species of *Cylindrocorpus* Goodey is given.

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TWO NEW SPECIES OF *PRATYLENCHUS*

BY

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Root rot in *Convallaria majalis* (lily of the valley) has long been associated with a species of *Pratylenchus* and SLOOTWEG (1956) showed that this species did not attack *Narcissus* (daffodils). Investigation of specimens from *Convallaria* showed that although they had characters in common with *P. penetrans* and *P. pratensis*, they belong to a new species described below as *P. convallariae*. SHER & ALLEN (1954) mention the finding of *P. pratensis* (de Man) in roots of *Convallaria* from Hamburg: this may have been a case of infection with *P. convallariae*.

The second new species, described below as *P. helophilus*, was abundant in a pasture on heavy clay soil at Wageningen. Nothing is known about its feeding habits.

Pratylenchus convallariae n. sp.
(Fig. 1 A, B)

Dimensions ♀ (n = 10) L = 0.58-0.61 mm, a = 23-27; b = 6-9; c = 17-28; V = $^{26\text{-}55}$ 78-81 $^{3.6\text{-}5.5}$ Stylet 16-17 μ
♂ (n = 10) L = 0.49-0.58 mm, a = 27-36; b = 5.2-6; c = 20-23; T = 25-51 %, Stylet 15-17 μ

Female (Holotype) L = 0.62 mm, a = 31; b = 7; c = 24; V = ^{44} 81 $^{3.6}$ Stylet = 17 μ

Cuticle marked by fine striae, about 1.5 μ apart. Lateral fields with 4 incisures. Lip region slightly set off from body and bearing 3 annules. Lateral margins of cephalic framework extending posteriorly for about half a body-annule. Spear 17 μ long. Dorsal oesophageal gland opening into lumen of the oesophagus about 3 μ from spear base. Excretory duct opening ventrally at level of junction between oesophagus and intestine. Hemizonid situated just anterior to this opening. Deirids not seen. Female tail sometimes bluntly rounded and with striations around the tip as in *P. pratensis* but mostly more or less split into two irregular lobes (see fig. 1 B). Phasmids at middle of tail. The inner two incisures of the lateral field go past the phasmid as in *P. penetrans*.

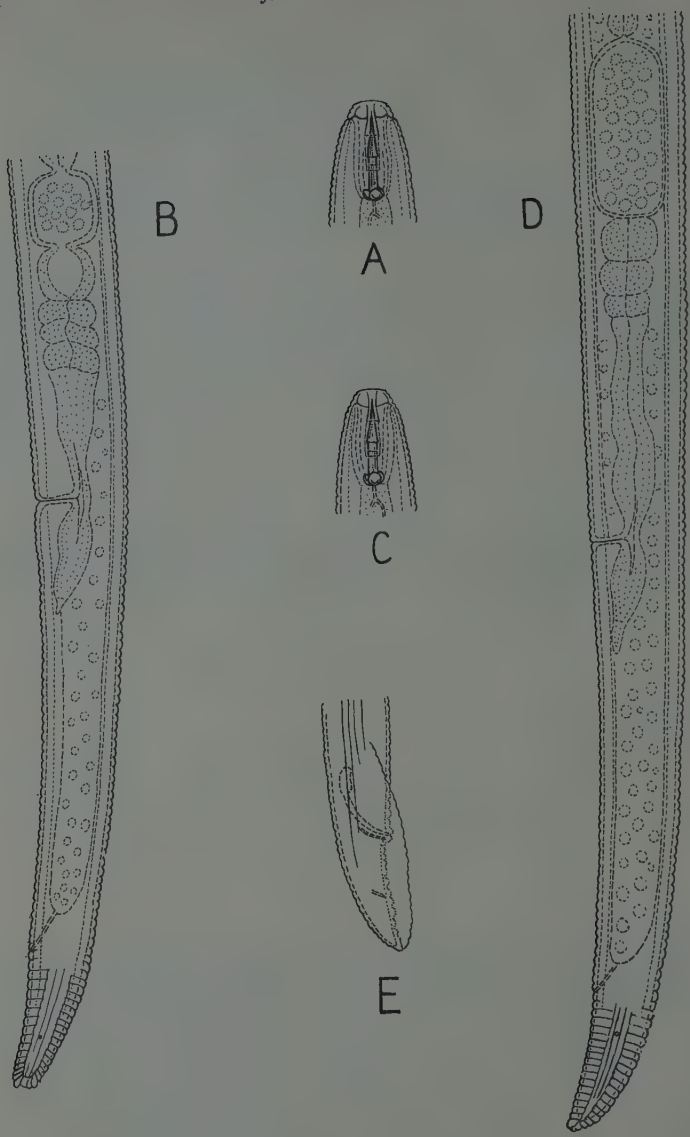

Fig. 1. Pratylenchus convallariae. A female head (800 x), B posterior part of female (800 x).
Pratylenchus helophilus. C female head (800 x), D posterior part of female (800 x), E male tail (800 x).

Ovary single, outstretched, with a single row of oocytes. Spermatheca round. Uterus short. Postvulvar uterine sac extending backwards 1.4-2 times the body width at the vulva and about as long as the tail.

Male (*Allotype*) — L = 0.52 mm, a = 30, b = 52, c = 23, T = 43 %
Stylet = 16 μ.

The male does not differ anatomically from the males of *P. penetrans*.
Holotype: Female collected from the roots of *Convallaria majalis*, no. TC 2/1 Institute for Phytopathological Research (I.P.O.) Nematode Collection.
Allotype: Male same data as holotype no. TC 2/2 I.P.O. Nematode Collection.
Paratypes: 44 females and 18 males same data as holotype no's TC 2/3, 4, 5 I.P.O. Nematode Collection.
Type host: Roots of *Convallaria majalis*.
Type locality: Wassenaar, Netherlands.
Relationships: The species resembles *P. penetrans* most closely, in size and form. It differs from this species in the tail tip being irregularly lobed and having incisures. The latter are less regular than in *P. pratensis* from which species *P. convallariae* is also different in having a spermatheca. *P. convallariae* causes lesions in the root cortex of *Convallaria majalis*; in heavy attacks the lesions may lead to a serious root rot. The species was also found in sandy soil at Wageningen and 's-Graveland (both in the Netherlands).

Pratylenchus helophilus n. sp.
(Fig. 1 C, D, E)

Dimensions ♀ (n = 10) L = 0.48-0.62 mm, a = 27-38, b = 5.0-7.1
c = 17-22, V = $^{32-44}$ 77-80 $^{3.9-5.3}$
Stylet 16-17 μ long

♂ (n = 10) L = 0.46-0.60 mm, a = 31-43, b = 5.4-8.1
c = 16-25, T = 36-61%
Stylet 14-16 μ long

Female (*Holotype*): L = 600 μ, a = 30, b = 5, c = 20, V = ^{37} 78 ^{5}
Stylet 16 μ long.

Body annules about 1.5 μ wide. Lateral fields with 4 incisures. Lips with 3 annules. Lateral margins of cephalic frame-work extending posteriorly for about half a body annule. Dorsal oesophageal gland opening into lumen of oesophagus about 3 μ behind spear knobs. Ex-

cretory pore ventrally at level of junction between oesophagus and intestine. Hemizonid just above excretory pore. Deirids not seen. Tail tip bluntly conical almost as in *P. vulnus* but with striations around the tip. Single outstretched ovary with double row of oocytes over most of its length. Spermatheca somewhat variable in shape but mostly oval as in *P. goodeyi*. Cellular oviduct rather short. Uterus about twice as long as body width at vulva. Postvulvar uterine branch about 1.5 times body width at vulva.

Male (*Allotype*) — L = 0.47 mm, a = 43; b = 6.1; c = 18; T = 36; Stylet 16 μ

Males of *P. helophilus* do not differ anatomically from males of *P. penetrans* and *P. vulnus*.

Holotype: Female, no. T C 3/1 I.P.O. Nematode Collection.

Allotype: Male no. T C 3/2 I.P.O. Nematode Collection.

Paratypes: 46 females and 14 males nos 3/3-4, 5, 6, 7 I.P.O. Nematode Collection.

Type habitat: Heavy clay soil under grass.

Type locality: Institute for Phytopathological Research, Wageningen, Netherlands.

Relationships: *P. helophilus* can be distinguished from other species with three annules on the lips by the large spermatheca, the long uterus and the rather pointed tail with faint annulation around its terminus.

It resembles *P. goodeyi* Sher and Allen in general shape and in the anatomy of the female reproductive system, but differs from it in the number of annules on the lips (4 in *P. goodeyi*, 3 in *P. helophilus*) and in the shape of the tail, which is less slender and has annulations around the terminus.

The author wishes to thank Mr A. F. G. Slootweg for sending the *Convallaria* roots in which *Pratylenchus convallariae* was found.

ZUSAMMENFASSUNG

Zwei neue *Pratylenchus*-Arten werden beschrieben. *P. convallariae* n. sp. verursacht die Fäule der Wurzeln von *Convallaria majalis*, das Maiglöckchen.

Die zweite Art *P. helophilus* n. sp. wird zahlreich gefunden in Wiesen auf schwerem Tonboden bei Wageningen.

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SCIENTIFIC NOTE

Vernon G. Perry, A Note on Digonic Hermaphroditism in Spiral Nematodes (*Helicotylenchus spp.*).

Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture [1]).

Reproduction by bisexuality, parthenogenesis, and hermaphroditism is known to occur among different species of Nematoda. Maupas (1900) described the types of reproduction for Nematoda and listed several protandrous hermaphroditic species. Cobb (1918) discussed hermaphroditism among certain nematodes and observed functional sperm in the gonads of some species in which males are either unknown or rare. In most cases he did not record the source of the sperm and applied the term "syngonic females" (sperm and ova produced in the same gonad). This is considered to be protandrous hermaphroditism. In *Mononchulus ventralis* Cobb found that functional sperm are produced in the small posterior gonad (testis) and applied the term "digonic females" (sperm and ova produced in different gonads of the same individual).

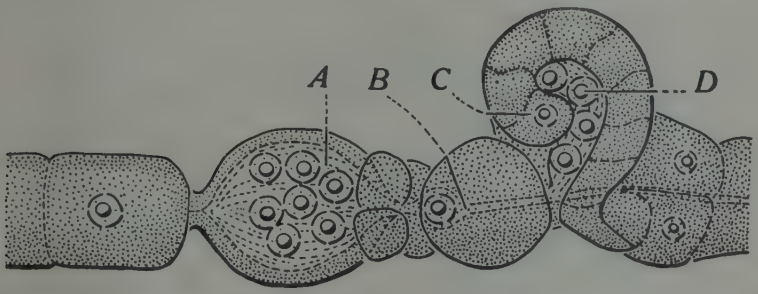

Figure 1. Spermagonium and spermatheca of hermaphroditic spiral nematodes, x 2200. A — spermatheca with sperm. B — oviduct with advancing sperm. C — primordial germ cell. D — sperm in spermagonium.

Functional males exist in many plant parasitic species, but in others males are unknown or so rare that parthenogenetic or hermaphroditic reproduction must occur. A member of the genus *Meloidogyne,* which can reproduce sexually, was shown by Tyler (1933) to reproduce without males in aseptic culture. Mulvey (1955) found that *Heterodera trifolii* reproduces by parthenogenesis.

A study of the gonads of certain spiral nematode species, which are probably monosexual since males have not been found in the examination of thousands of specimens, revealed the presence of a spermatheca adjacent to the entrance of the oviduct in which well developed spermatozoa were usually present. Further observations revealed that the spermatozoa originated in a spheroid structure located dorsally from the oviduct near its junction with the spermatheca.

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[1]) In cooperation with Department of Plant Pathology, Wisconsin Agricultural Experiment Station, University of Wisconsin, Madison, Wisconsin, U.S.A.

This sperm-producing organ, Figure 1, contains the primordial germ cell (C) in which a prominent nucleus may or may not be observed in formalin fixed specimens, depending on the phase in which it happens to be when the specimen is killed. This cell apparently produces a primary spermatocyte by mitosis which then becomes four spermatozoa by meiosis (D). These, without further division, move through the oviduct (B) to the spermatheca (A) where they await and fertilize the oöcytes.

As the oöcyte enters the spermatheca it changes from a nucleated cell to a granular mass in which the nucleus is usually not visible and doubtless is fertilized during this stage. As it passes through the oviduct after fertilization the egg shell is laid down and it emerges into the uterus ready for deposition.

These structures were repeatedly observed in *Helicotylenchus nannus* Steiner 1945, and three other undescribed species of spiral nematodes. They are believed to be always present in these species, but are often difficult to observe and not previously observed because they are usually obscured by an oöcyte at the entrance of the oviduct. According to Cobb (1918) these individuals are "digonic females" or "digonic hermaphrodites", since they possess two distinct reproductive organs in each reproductive system. The term "spermagonium" is suggested as a name for the sperm-producing structure, Figure 1. Spermagonium is, according to Webster's dictionary (1954), an obsolete term but it has been used as a synonym for the botanical term spermagonium.

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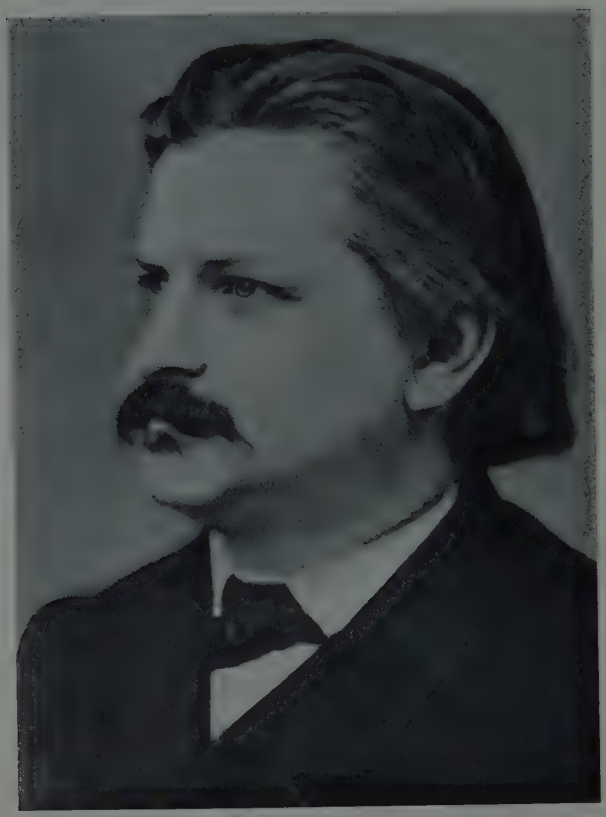

HERMANN SCHACHT, 1859-1864 Ordinarius für Botanik in Bonn.

Die Aufnahme wurde vom Botanischen Institut der Universität Bonn freundlichst zur Verfügung gestellt.

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100 JAHRE *HETERODERA SCHACHTII*

VON

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Im Jahre 1859 beobachtete HERMANN SCHACHT, der von 1859-1864 Ordinarius für Botanik an der Universität Bonn war, in der Umgebung von Halle (Saale) an den Wurzeln der Zuckerrübe kleine weisse Körperchen von der Grösse eines kleinen Stecknadelkopfes, die er als die Weibchen eines Nematoden deutete. In seiner ersten Mitteilung hierüber gibt SCHACHT bereits eine genaue Beschreibung des Weibchens und der Eier und fügt auch Abbildungen bei, die jeden Zweifel an der richtigen Deutung ausschliessen. In Würdigung der Verdienste SCHACHTS um die Entdeckung des Rübennematoden hat A. SCHMIDT ihn 1871 *Heterodera schachtii* genannt.

Seit dem Bekanntwerden des Rübennematoden ist bis zum heutigen Tage eine Unzahl wissenschaftlicher und populärer Abhandlungen erschienen. *Heterodera schachtii* wurde geradezu zum Muster pflanzenparasitärer Nematodenuntersuchungen und zur Stammform aller nachher bekannt gewordener zystenbildender Nematoden, die meistens zunächst als Varietäten von *H. schachtii* beschrieben, später aber als eigene Arten erkannt wurden. Erstmalig wurde auch nachgewiesen, dass die als „Müdigkeit" bezeichneten Symptome der Zuckerrübe mit dem Befall durch *Heterodera schachtii* in ursächlichem Zusammenhang stehen.

In der Geschichte des Rübennematoden lassen sich zwei Höhepunkte besonders intensiver Bearbeitung unterscheiden. Der erste Höhepunkt umfasst die Zeit von 1878 bis 1908, in der Männer wie LIEBSCHER, KÜHN, HOLLRUNG, WILLFARTH, ROEMER und WIMMER sich mit praktischen Problemen des Rübennematoden befassten. In diesen Zeitabschnitt fällt auch die klassische Veröffentlichung STRUBELLS (1888), in der Bau und Entwicklung des Rübennematoden in monographischer Weise behandelt werden. Der zweite Höhepunkt wird durch die Untersuchungen BAUNACKES (1922) eingeleitet, der richtungweisende Arbeiten zum Problem der Wirtsauffindung durch den Nematoden und

zur Aktivierung seiner Larven durchführte. Im Zuge dieser Beobachtungen erschienen zahlreiche Veröffentlichungen, die eine künstliche Aktivierung mit chemischen Mitteln zum Ziele hatten (RENSCH, NEBEL, HOMUTH, HORNBURG, MOLZ, RADEMACHER, O. SCHMIDT und GOFFART). Vorherrschend waren namentlich die reizphysiologischen Untersuchungen, die auch in unseren Tagen noch keineswegs abgeschlossen sind, wenn sie auch durch das inzwischen aktuell gewordene Kartoffelnematodenproblem etwas zurückgedrängt wurden.

Andere um 1930 eingeleitete Untersuchungen behandeln die Rassenfrage und grenzen den Wirtspflanzenkreis von *Heterodera schachtii* von den Kreisen anderer *Heterodera*-Arten ab.

Die wirtschaftliche Bedeutung des Rübennematoden wurde schon zu KÜHNS Zeiten mit Interesse auch im Ausland verfolgt, wie die Arbeiten GIRARDS aus den Jahren 1884-1887 und CHATINS aus den Jahren 1887-1891 zeigen.

Nach dem ersten Weltkrieg fand *Heterodera schachtii* in allen Rübenbau betreibenden Ländern grosse Beachtung. Hier sei nur an die Namen HELLINGA (Holland), TRIFFITT (England) und THORNE (USA) erinnert, denen sich sehr bald eine grosse Zahl weiterer Namen anschliesst. Heute hat das Rübennematodenproblem in Europa nicht mehr die praktische Bedeutung als ehedem. Wir haben es gelernt, mit dem Rübennematoden zu wirtschaften und halten ihn hauptsächlich durch Fruchtfolgemassnahmen in Grenzen. Dennoch spielt *Heterodera schachtii* in der Nematologie, namentlich bei Untersuchungen über das Aktivierungs- und das Resistenzproblem, auch heute noch eine beachtliche Rolle.

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RESISTANCE OF BARLEY TO VARIOUS POPULATIONS OF THE CEREAL ROOT EELWORM (*HETERODERA MAJOR*)

BY

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The first evidence of resistance to the cereal root eelworm (*Heterodera major* O. Schmidt = *H. avenae* Filipjev) was shown at Svaløf as early as 1920 (NILSSON-EHLE 1920). He found that eelworm cysts failed to develop on the roots of three barley (*Hordeum sativum* L.) varieties, Primus, Svanhals, and Chevalier. In several trials these varieties were completely without cysts. Later on the resistant Danish varieties Drost, Fero, and Kron were bred (BØGH 1951 and 1952), and in the writer's tests at Albertslund resistance was found in the variety Alfa. In these tests however, resistance was not complete. BØGH found a small number of cysts on the roots of the resistant varieties, and at Albertslund cysts were found on Drost, Fero, Kron, and Alfa. Also the varieties Primus and Svanhals bore some cysts at Albertslund.

In 1947-49 some varieties were tested at Lyngby by the late K. J. RASMUSSEN (ref. by ANDERSEN 1951), but in these trials the above mentioned varieties were found to be susceptible. Due to these conflicting results it was decided to test a number of resistant varieties on various infected soils.

In the trial by JØRGENSEN & THOMSEN (1928) only a small number of cysts was formed on the Danish variety Rex, and the yield of this variety was rather high on infected soil. A selection, Rex II, was used as a check variety in the writer's field trials, but the eelworm produced nearly half as many cysts on this selection as on Maja and Herta (Table I).

PATHOGENICITY OF THE LYNGBY AND ØDUM EELWORM POPULATIONS

In 1955 a preliminary test was carried out on various infected soils, and in the next year 72 varieties were sown on infected soil in five

localities. 61 of these varieties had shown resistance at Albertslund. The five localities were Hasle and Ødum near Aarhus, where the soil is a clay loam with a clay subsoil, and at Lyngby, Højbakke, and Svogerslev near Copenhagen. The soil at Lyngby and Svogerslev is a light clay loam over sandy clay subsoil; a similar soil occurs at Højbakke but the content of organic matter is higher.

The trials were carried out in the field in small plots measuring one by two metres. Within each of these plots eighteen varieties and a duplicate of the check variety were sown. Three seeds of each variety were sown at ten points within the plot, and these 200 'microplots' were arranged in a Latin Square. The area occupied by 30 plants of each variety was 0.1 square metre. In the middle of July, all the plants were lifted, and the cysts were counted when the roots had been washed. At the two places near Aarhus the number of cysts was high enough to make a good classification possible, but at the other places the number of cysts was rather low.

TABLE I

Susceptibility of ten barley varieties grown in five infected fields. Number of cysts on the roots of 30 plants. Experiment in 1956.

Variety	Place of resistance trials				
No. and name	Lyngby	Højbakke	Svogerslev	Hasle	Ødum
429. Rex II (check)	19	37	33	71	186
295. Herta	83	78	60	140	235
304. Maja	52	57	35	200	345
433. Drost	68	0	0	2	202
254. Alfa	15	1	0	2	272
276. Fero	65	0	0	4	206
429. Rex II (check)	63	31	22	244	153
291. *Hordeum distichum*	9	0	0	7	3
191. *Hordeum pallidum*	0	1	0	0	2
14. Barley 16	3	0	0	3	15
302. Kron	10	0	2	22	12

In Table I figures are given for ten varieties. Herta, Maja, and the check variety Rex II belong to a group of varieties which are susceptible at all five localities. Another group, represented by Drost, Fero, and Alfa, are varieties which are susceptible at Ødum and Lyngby, but resistant at Hasle, Svogerslev, and Højbakke. This group consists of nearly fifty varieties. Thirty eight of these had less than five cysts

at Hasle, Svogerslev, and Højbakke, (total for all three places), but more than 100 cysts at Lyngby and Ødum. They are not listed, but further information about the resistance of these and other varieties may be had on request to the writer. Seeds can also be supplied.

Barley collection no. 191 was resistant at all five places. Other resistant varieties were Kron and coll. no. 14 and 291, but only these four varieties showed resistance to the Ødum and Lyngby population.

The different pathogenicity of various eelworm populations might be due to environmental factors and not to the eelworm itself. In order to make a test under equal conditions, soil from the infected fields, except Højbakke, was brought to Albertslund and used for resistance trials there. Only four varieties were tested, Svaløf Sun II oat, Abed Maja barley, and the two resistant Danish barley varieties Drost and Alfa. The experiment was carried out in clay-pipes of the type normally used for drainage. Pipes 2½ inches in diameter were sunk into the ground and filled with soil, three seeds were then sown at the top.

In 1956 each variety was sown in twelve pipes. Four pipes were filled with infected soil with an infestation level of about 7000 eggs and larvae per kg of soil, and eight pipes were filled with cyst-free soil. Cysts, which were obtained by floatation of wet soil in a Fenwick-can, were added to the cyst-free soil. A float which consisted of cysts and organic debris obtained from 600 grammes of soil was used for infection of each of four pipes. Twenty five full, clean, hand-sorted cysts were used for each of the last four pipes.

In 1957 the experiment was carried out with clean cysts only. In that year each barley variety was sown in six pipes, but the oat variety Sun II was not tested. In both years the cysts on the roots of the plants were counted in the middle of July.

The result of the tests was the same whether infected soil, concen-

TABLE II

Tests of three barley varieties against four populations of the cereal root eelworm. Total number of cysts from plants grown in twelve clay-pipes in 1956 and six pipes in 1957.

Variety	Soil or cysts from			
	Lyngby	Svogerslev	Hasle	Ødum
Maja	1103	501	973	883
Drost	463	0	3	657
Alfa	486	0	2	696

out at Rothamsted, showed that agressive populations were common in English soils (JONES 1957, 1958).

The term 'agressive' was used by DUNNETT to 'denote an increase in the facility with which the parasite overcomes resistance, and reproduces in the host' (DUNNETT 1957). DUNNETT based his use of the term on the definition by GÄUMANN (1946). The term 'biotype' has been used to denote agressive individuals of the potato root eelworm (DUNNETT 1957, JONES 1957). As these two terms are accepted in the case of the potato root eelworm, they will also be used in the present discussion.

By chance the first test for resistance to the potato root eelworm seems to have been done on soils with a non-agressive biotype, and in the case of the cereal root eelworm the situation is similar. The pioneer work of NILSSON-EHLE (1920) must have been done on a soil containing a pure non-agressive population, and the breeding work by BØGH (1952) has been carried out on a soil, where agressive individuals were not dominant.

The present work has shown the presence of agressive and of pure non-agressive populations. Intermediate populations have also been found. Such intermediate populations also occur in the case of the potato root eelworm, and they may be a mixture of a non-agressive biotype and one or more agressive ones (JONES 1957), but other explanations are possible. The intermediate populations may consists of one or more biotypes with a medium pathogenicity, or the biotypes may interbreed and produce heterozygous individuals. Further investigations may solve these problems, but it seems likely that there are at least two biotypes, a non-agressive and an agressive one.

Those thirty six populations, which did not produce cysts on Drost and Alfa, must consist of pure non-agressive individuals, or individuals of the agressive biotype must at least be very uncommon. Continued growing of Drost or Alfa in fields with such populations would quickly reduce the infestation level of the soil. But some populations produced a very small number of cysts on Drost and Alfa. If those few cysts are individuals from the agressive biotype, growing of Drost or Alfa or other varieties resistant to the non-agressive biotype only, would change the population to a more agressive one.

The number of cysts produced on no. 191 was generally small. Eighty four populations did not form cysts on this variety at all, and thirteen populations produced less than eight cysts. But two populations produced a considerable higher number of cysts on no. 191. The number

was twenty seven and forty five respectively, and the same two populations produced 190 and 342 cysts on Herta. These figures make it probable that a biotype adapted to no. 191 may be found, but it has not been settled whether the cysts formed on no. 191 really are cysts of *H. major*. The populations must be re-tested before any conclusion can be reached. The figures could also be explained if we suppose that resistant varieties are not resistant under all growing conditions.

Differences in resistance have been found so far only in trials with barley. In oats (*Avena sativa* L.) the number of resistant varieties is smaller than in barley, but oat varieties which up to now have shown resistance are all resistant to both biotypes. None of these varieties is completely resistant, but plants with one or two cysts are found more frequently in oats than in barley. The resistance genes of oats are obviously less effective than those of barley, and some nematodes may happen to overcome the resistance barrier of the plant. *Avena sterilis* was tested for resistance to the same ninety nine populations as barley and eighty populations did form cysts on this species. There was no correlation between pathogenicity to barley and pathogenicity to *A. sterilis* but cysts on *A. sterilis* were developed by agressive as well as non-agressive populations. The number of cysts produced on *A. sterilis* was generally below 5% of the number of cysts produced on Sun II. The highest number of cysts produced on *A. sterilis* by one population was 38, but the same population produced 800 cysts on Sun II.

The terms agressive and non-agressive are misunderstood by some people, who consider an agressive biotype as a type which does more harm to the plant than a non-agressive one. Other difficulties would arise, if more biotypes should be found. In order to avoid these troubles, the biotypes could be given a number. In that case the non-agressive type of the cereal root eelworm must be named as race or biotype no. 1, as the original work by NILSSON-EHLE was done on a soil containing a non-agressive biotype. The term biotype no. 2 must be given to a biotype which produces cysts on Drost and Alfa in approximately equal numbers as on the varieties Herta and Maja.

Acknowledgement. I wish to thank Mr van Hiele, Laboratorium voor Landbouwplantenteelt, Wageningen, the Netherlands, who kindly supplied me with seed of *Avena sterilis*.

ZUSAMMENFASSUNG

Resistenz von Gerste gegenüber verschiedenen Populationen des Haferälchens (Heterodera major)

Zwei biologische Typen des Hafernematoden (*Heterodera major*) wurden gefunden. Viele Populationen des Älchens sind nicht imstande, Cysten an den Gerstensorten Drost und Alfa zu bilden. Andere Populationen jedoch enthalten aggressive Individuen, die in der Lage sind, die Resistenz dieser beiden Sorten zu brechen. Boden von 99 infizierten Feldern wurde untersucht. In mehr als 50 Böden trat der aggressive Biotyp auf.

Eine Gerstensorte, No. 191, ist resistent gegenüber beiden Biotypen. Das etwaige Vorkommen eines dritten Biotyps, der an diese Sorte angepasst ist, wird besprochen.

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HOPLOLAIMUS ANGUSTALATUS N.SP. (HOPLOLAIMINAE: TYLENCHIDA)

BY

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During a survey of nematode attacks in the crop plants of the Southern Highlands Province, Tanganyika Territory, the writer found roots of banana plants at Makete and Rutengano, Rungwe district to be swollen and cracked. At first this was thought to be due to the invasion of the roots by a species of the nematode genus, *Meloidogyne* Goeldi but further examination revealed heavy populations of a new species of *Hoplolaimus* von Daday, 1905 in affected roots. SHER (1954) reported the occurrence of numerous specimens of *Hoplolaimus* sp. around declining banana plants in Hawaii. While the roots of banana plants are widely attacked in East Africa by species of *Pratylenchus* Filipjev, 1934, *Meloidogyne* Goeldi, 1887 and *Rotylenchus* Filipjev, 1934, invasions of *Hoplolaimus* spp. are not common. This is the first fully identified species of *Hoplolaimus* to be recorded as a parasite of the banana (*Musa paradisiaca* L. v. *sapientum*) and the first record of such an attack in East Africa.

DESCRIPTION OF THE DISEASE

While a few specimens of *Hoplolaimus angustalatus* were found with an ecto-parasitic habit, the head being imbedded in the root cortex and the rest of the body lying outside the root, the majority of the large populations found were endoparasitic.

The earliest visible root symptom is the appearance of dark brown, narrow pustules about 1 or 2 mm long on the surface of the root, which lie parallel with the root axis. These pustules contain specimens of *H. angustalatus* and owe their dark brown coloration to the cortical necrosis, which arises in the vicinity of the head of each nematode. As the nematodes invade the deeper cortical tissue, the necrosis becomes more extensive and may develop along the length of the invaded root. The pustule distintegrates giving rise to an elongate, ulcerated lesion, which

enlarges with the growth in girth of the affected root (Fig. 1). The histopathology of this disease will be published later.

DESCRIPTION OF THE PARASITE

(Figs. 2 and 3)

Hoplolaimus angustalatus n. sp.

Diagnosis. — Hoplolaiminae. Head with six lips without papillae; four or five annules at least three of which divided longitudinally into numerous minute blocks, lowest annule bearing about twenty. Guide ring surrounds fore part of spear. Basal knobs of spear each bear three to five "teeth" on anterior margin. Body annules interrupted by vestigial lateral fields. Large posterior oesophageal region. Excretory pore situated opposite junction of isthmus with posterior region of oesophagus and on ventral surface, while hemizonid lies about eight annules posterior to excretory pore. Male only slightly shorter than female. Gubernaculum non-cephalate distally and without titillae. Telamon not observed.

DIMENSIONS

18 ♀♀: L = 1.045 — 1.620 mm ($\bar{x}$ = 1.399 ± 0.028)
a = 20 — 30
b = 4.2 — 8.5
c = 52 — 89
V = 51.1 — 77.5%

Body width at oesophago-intestinal junction
= 0.045 — 0.055 mm ($\bar{x}$ = 0.048 ± 0.010)

Body width at anus = 0.031 — 0.039 mm ($\bar{x}$ = 0.035 ± 0.01)

14 ♂♂: L = 1.261 — 1.467 mm ($\bar{x}$ = 1.328 ± 0.020)
a = 25 — 31
b = 5.3 — 8.2
c = 34 — 48

Length of spicule = 0.044 — 0.057 mm ($\bar{x}$ = 0.051 ± 0.001)
Length of gubernaculum = 0.021 — 0.026 mm ($\bar{x}$ = 0.023 ± 0.0004)
Length of bursa = 0.067 — 0.088 mm ($\bar{x}$ = 0.077 ± 0.002).

Holotype ♀ L = 1.423 mm; a = 23; b = 7.1; c = 89; V = 56.8%.
Allotype ♂ L = 1.405 mm; a = 28; b = 5.9; c = 43.

Fig. 1. Banana root under high magnification, showing open lesions caused by *H. angustalatus*.

MORPHOLOGY

Head has hemispherical shape in both sexes, ratios head width : head length for female and male respectively being 2.0 — 2.6 ($\bar{x}$ = 2.177 ± 0.042) and 1.8 — 2.2 ($\bar{x}$ = 1.960 ± 0.040).

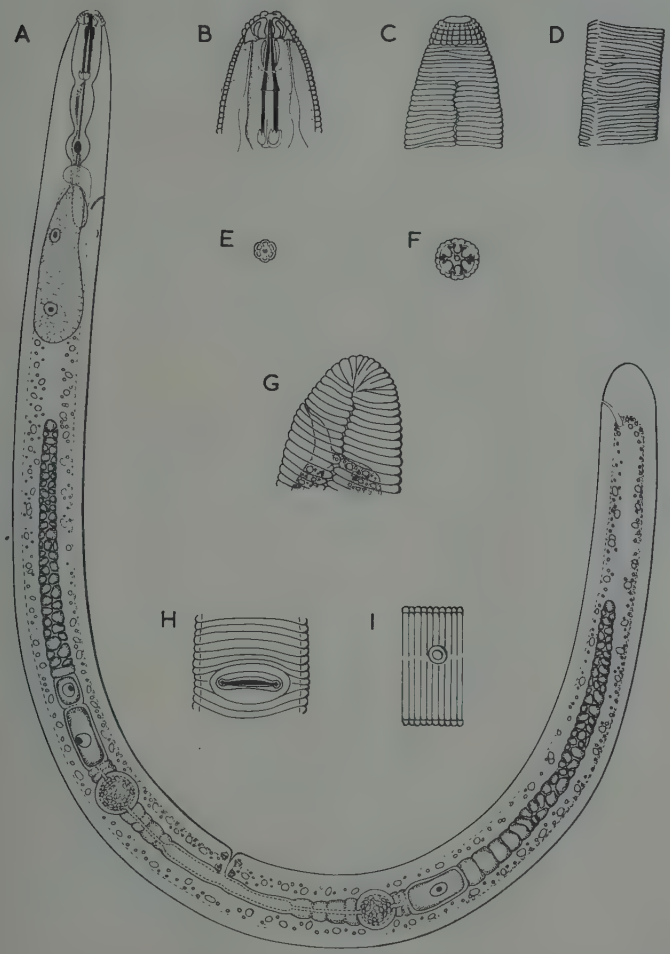

Fig. 2. *Hoplolaimus angustalatus*. A ♀; B head end; C head end, lateral surface view; D. lateral field; E *en face* view of head; F. basal plate of head; G. tail end; H vulva in ventral view; I phasmid. (For magnification see scales on Fig. 3 — scale 1 applies to A, scale 2 to the rest).

Head has six lips, surmounted by central head-cap. Lips bear no papillae and lateral lips, on which amphidial apertures could not be seen, are slightly narrower than subdorsal and subventral lips, which are approximately equal width. There are four or five head annules, although one female was observed to bear six. Lowest annule divided up by some twenty longitudinal striae, which similarly divide next two annules and there are traces of division visible on fourth and, if present, fifth annule.

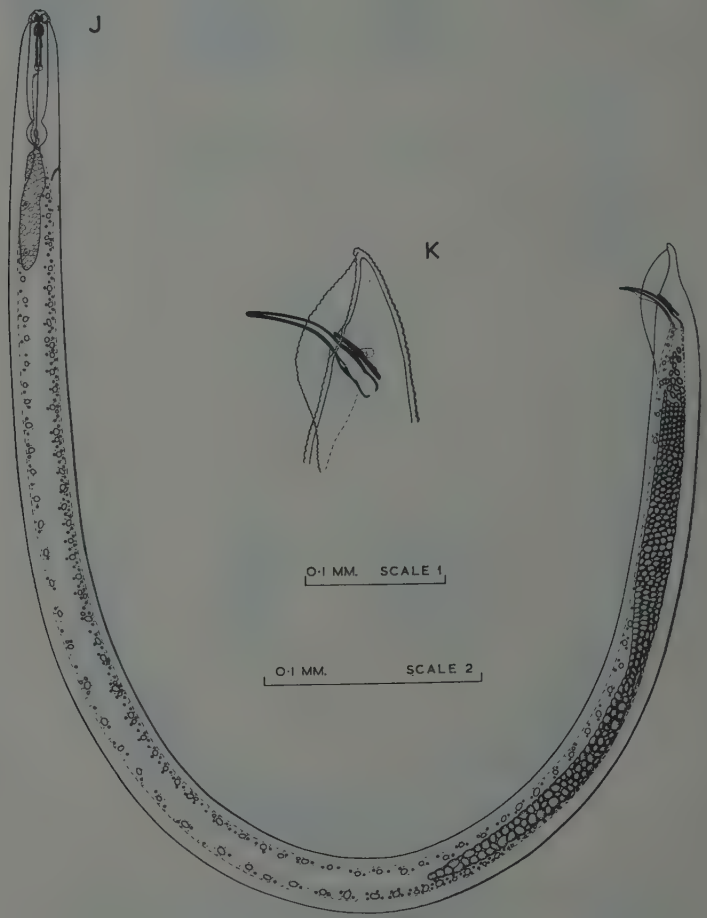

Fig. 3. *Hoplolaimus angustalatus*. J ♂ ; K tail end. (Scale 1 applies to J, scale 2 to K).

Skeletal framework of head arises from basal plate composed of six arms fused centrally around vestibule. Dorsal and ventral arms of this plate have two larger proximal and two smaller distal branches, latter arising just behind lip margins. Lateral arms bifurcated at lip margins. Walls of vestibule, through which spear passes strengthened by thickened skeletal tube arising from basal plate and from this a shield supports sides of head and rejoins basal plate. Immediately behind basal plate lumen of fore part of corpus is strengthened by cuticular tube while guide ring emerging from basal plate passes behind this tube and around spear. Two backward extensions from guide ring reach back to junction of fore and aft regions of spear.

Spear massive measuring 38 μ — 48 μ in length ($\bar{x} = 44.82 \pm 0.54$) in both sexes. It is composed of a tapering anterior region, spear aperture subterminal and ventral, a cylindrical shaft of same length and at base of shaft three knobs, each of which carries from three to five "teeth" in its anterior margin. Lateral margins of spear knobs carry minute processes which, as well as the "teeth", serve as muscle attachments.

Oesophagus composed of cylindrical corpus behind which is a median bulb with crescentic valve and muscular walls. Isthmus surrounded by the circum-oesophageal nerve ring and connecting the median bulb to glandular posterior oesophageal region. This glandular region is larger than normal for *Hoplolaimus* spp., especially in female, and is largely made up of dorsal oesophageal gland cell and two much smaller subventral gland cells, which are largely hidden by the dorsal cell. Dorsal oesophageal gland duct opening into lumen of the corpus about half a spear's knob length behind spear, while ventral gland duct appear to open into lumen of median bulb immediately behind crescentic valves.

Intestine is plain, thick-walled tube whose cells are full of fat granules and which opens by a thin walled rectum at anus.

Annulations of cuticle are about 2 μ wide and 1 μ wide on subcuticle. Except for some ten annules behind head, striae are interrupted laterally thus forming a rudimentary lateral field and it is this character to which the Latin epithet — *angustalatus* — refers (Fig. 2, C, D and G). On tail of female striations tend to fuse before reaching lateral field (Fig. 2 G). Striations on male tail are interrupted at point of origin of bursal wing.

Excretory pore lies on ventral body surface behind median bulb a distance varying from nil to 0.045 mm ($\bar{x} = 0.021 \pm 0.003$) in the female and 0.010 — 0. 048 mm ($\bar{x} = 0.021 \pm 0.004$) for males. Hemi-

zonid lies 3 — 9 annules behind excretory pore in female worms and 4 — 12 for males.

Phasmids large sub-spherical sacs situated erratically. In female, anterior phasmid is situated from 25% to 40% of body length, while posterior phasmid situated from 75% to 85% of body length. Anterior phasmid may be on right of body and posterior on left or vice versa. In male, anterior phasmid is on right side from 30% to 50% of body length, while posterior phasmid is on left side from 60% to 80% of body length.

Unlike *H. proporicus* Goodey, 1957, body of *H. angustalatus* is translucent.

Female reproductive organs paired, equal and generally outstretched, although one specimen exhibited ventral flexure of anterior ovary. Ovaries long and reach far forwards and backwards in body. Spermathecae spherical sacs containing spermatozoa and lead to uterus, which is thick walled for half its length and then passes into a thin walled sac, which leads to muscular, thick walled vagina which in turn leads to a wide transverse vulva.

Single testis and vas deferens in male. Copulatory bursa arises at level of spicule heads and passes back to tail tip. Spicules are large, with open heads, short straight-sided shafts and slender blades from each of which arises a lateral flange in median plane. Gubernaculum is a simple plate with slight thickenings dorsally about middle and at proximal end. No titillae but a lightly cuticularised lobe is apparently attached to dorsal surface of gubernaculum.

Type specimens — Holotype ♀ No. 6 slide M.s. 2, Allotype ♂ No. 4 slide M.s. 3. in slide collection of the Division of Nematology, East African Agriculture and Forestry Research Organisation, P.O. Box 21, Kikuyu, Kenya.

Type host — Found within the roots of banana plant (*Musa paradisiaca* L. var. *sapientum*).

Type locality — Makete Village, Rungwe District, Southern Highlands Province, Tanganyika Territory.

DISCUSSION

Hoplolaimus angustalatus differs from its nearest relative. *H. proporicus* in several respects — annules interrupted laterally to form very narrow lateral field; excretory pore located more posteriorly and always behind posterior margin of median bulb; hemizonid located about

eight annules behind excretory pore; posterior oesophageal region is much larger; head bears four or five annules lower three at least being extensively 'tiled'; male only slightly smaller than female; gubernaculum has thicker proximal half and bears no titillae; female tail is not abbreviated as occurs in some specimens of *H. proporicus*. Body of *H. angustalatus* not opaque as is that of *H. proporicus*.

Acknowledgements. The author is grateful to Mr. F. G. W. Jones and Dr. J. Basil Goodey of the Nematology Department, Rothamsted Experimental Station, England, for valued criticism of the text.

RÉSUMÉ

L'auteur donne une description de *Hoplolaimus angustalatus* n. sp., parasite des racines des bananiers.

Cette espèce diffère des autres espèces de ce genre par la possession de membranes latérales rudimentaires, une excroissance située entre la lunule du bulbe median et la région postérieure oesophagienne, la division des 3 anneaux basaux de la tête en secteurs et par l'absence de „titillae" sur le gubernaculum testis.

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BUNONEMA (RHODOLAIMUS) ESTONICUM N. SP. — EINE FAULENDE KARTOFFELKNOLLEN BEWOHNENDE NEUE NEMATODENART

VON

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Bei Untersuchungen der faulenden Kartoffelknollen auf ihre Nematodenfauna in Estland wurde eine Nematodenart aus der Unterfamilie Bunonematinae festgestellt, die mit keiner bisher bekannten Art identifiziert werden konnte.

Die neue Art gehört in die Fuchs'sche Gattung *Rhodolaimus* Fuchs, 1930, welche nach der eingehenden Revision von Sachs (1949) nur für eine Untergattung der Gattung *Bunonema* Jägerskjöld, 1905 gilt. Sie ist zwar *Bunonema* (*Rhodolaimus*) *poligraphi* (Fuchs, 1930) Sachs, 1949 am nächsten verwandt, aber nähert sich nach den Flossenbildungen etwa einer Art aus der Untergattung *Stammeria* Sachs, 1949. Diese Tatsache zeigt nochmals, dass die Untergattungen von *Bunonema* einander sehr nahe verwandt sind und deshalb nicht als selbständige Gattungen aufgestellt werden dürfen.

Beschreibung der Art (Abb. 1 a-e) :

Masse: 3 ♀♀	♂♂
Länge = 0,288- 0,473 mm	unbekannt
a = 9,4 -14,2	
b = 3,5 -4,0	
c = 13,4 -19,2	
V = 55,6 -58,5 %	

Kutikula und Kopf asymmetrisch ausgebildet. Mundhöhle zylindrisch, lang und schmal. Länge der Mundhöhle 11,5 μ. Übergangsstelle des Metastoms gut ausgebildet. Oesophagus typisch gestaltet, rhabditoid. Auf der linken Seite des Körpers sind 5 Kriechleisten vorhanden. Auf der rechten Seite liegen dorso- und ventrolateral zwei Warzen-

reihen. Warzenreihen am Vorder- und Hinterende in einzelne Flossen aufgelöst. Es sind zwei verschiedene Typen von Flossen ausgebildet. Die ersten vier Paare gut entwickelt, nur aus wenigen grossen Warzen bestehend, miteinander mit einer zarten Membran verbunden. Die folgenden zwei bis fünf (meistens drei bis vier) Flossen — man kann sie als „Übergangsflossen" bezeichnen — sind nicht höher als die Warzenreihe selbst. Dagegen sind sie aber erheblich länger und von 6-10 einzelnen Warzen (Lamellen) zusammengesetzt. Auch diejenige Flossen sind miteinander verbunden und gehen endlich ununterbrochen in

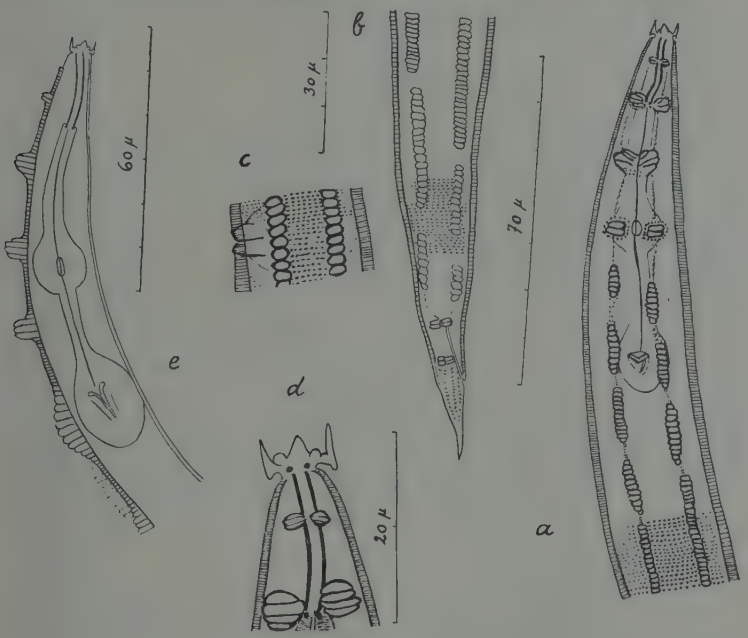

Abb. 1. *Bunonema (Rhodolaimus) estonicum* n. sp.
a Vorderende lateral; b Schwanzende lateral; c Vulva; d Kopf lateral; e Vorderende dorsal.

die Warzenreihe über. Es ist charakteristisch, dass die letzten Flossen am Vorderende nicht mehr paarweise gestellt sind, sondern sie stehen auf Lücke, wie bei *Bunonema (Stammeria) jakobii* Sachs, 1949. Warzenreihen am Körperende wieder aufgelöst. Auch hier sind die ersten 1-2 Flossen sehr lang, die letzten 2-3 aber bestehen nur aus wenigen Warzen.

Bei dem typischen Exemplar sind die Massangaben der Flossenbildungen folgende (L = Länge, B = Breite):

Mundhöhlenbeginn bis Flossenbeginn — 6 μ
1. Flosse: L — 2 μ, B — 2,5 μ
Abstand der 1. Fl. zur 2. Fl. — 6 μ
2. Fl.: L — 4,5 μ, B — 6 μ
2. Fl. bis 3. Fl. — 8 μ
3. Fl.: L — 6 μ, B — 5,5 μ
3. Fl. bis 4. Fl. — 10,8 μ
4. Fl.: L — 4 μ, B — 6 μ
4. Fl. bis 5. Fl. — links — 6 μ
rechts — 8,8 μ
5. Fl. links: L — 7,5 μ, B — 3,5 μ
rechts: L — 10,8 μ, B — 3,5 μ
usw.
Die letzten Flossen am Vorderende:
7. Fl. rechts: L — 11,75 μ, B — 3,5 μ
8. Fl. links: L — 13,7 μ, B — 3,5 μ

Am Hinterende:
Die erste abgelöste Fl. (links): L — 28,5 μ
2. Fl.: L — 14,7 μ
3. Fl.: L — 2 μ
4. Fl.: L — 2 μ

Kutikularstruktur besteht aus regelmässigen Querreihen kleiner Pünktchen. Zwischen den Warzenreihen sind die Punkte gröber.

Diagnose: Eine *Bunonema*-Art, die *B.* (*Rhodolaimus*) *poligraphi* (Fuchs, 1930) Sachs, 1949 morphologisch am nächsten steht; sie unterscheidet sich aber von dieser durch eine andere Gestaltung der Flossen (Vorhandensein von 2 Typen, die letzten am Vorderende stehen auf Lücke). Die Warzenreihe löst sich auch am Hinterende in Flossen auf. Eine Verbindung zwischen den Flossen ist vorhanden. Die Kutikularstruktur ist eine andere.

Endlich unterscheidet sich die neue Art durch eine andere Lebensweise, denn sie ist nicht mit den Borkenkäfern verbunden, sondern führt eine saprobe Lebensweise, wie auch einige andere Vertreter dieser Gattung.

Die Kutikularbildungen sind bei den *Rhodolaimus*-Arten zwar variabel — sie können manchmal schwach ausgebildet sein oder sogar fehlen. Bei der neuen Art sind diese Bildungen im Gegenteil gut entwickelt und kompliziert.

Typus: Weibchen No 5 (13) in der Kollektion des Institutes für Zoologie und Botanik der Akademie der Wissenschaften der Estnischen SSR. Paratypi: 10 Exemplare (4 ♀♀ und 6 Larven).

Typischer Fundort: Mustjärve (Bezirk Kallaste, Estland) am 26. Juni 1957. Eingelagerte Kartoffelknolle mit weitentwickelter Trockenfäule (im Keller). Fauna: *Diplogaster* sp., einzelne *Aphelenchoides parietinus, Aphelenchoides* sp., *Rhabditis curvicaudata, Acrobeloides* sp. und mehrere Exemplare von *B.* (*Rh.*) *estonicum* n. sp. — also nur wenige Arten.

Weitere Fundorte: Kanepi (Bezirk Pylva) am 18. März 1957. Faulende Knolle (Trockenfäule?) im Keller. Fauna: *Neotylenchus abulbosus*, weiter *Diplogaster sp., Tylopharynx* sp. und 1 Exemplar von *Bunonema* (*Rh.*) *estonicum* n. sp.

Dies ist der erste Fund von Subgenus *Rhodolaimus* in der UdSSR.

SUMMARY

Bunonema (*Rhodolaimus*) *estonicum* n. sp., from rotting potato tubers in Estonia is described. The new species is nearest to *B.* (*Rh.*) *poligraphi* (Fuchs, 1930) Sachs, 1949, but differs in having a more complex system of tubercles. The rows of warts terminate anteriorly and posteriorly in individual tubercles. The tubercles are held together by a delicate membrane. The posterior two to five tubercles on the anterior part of the body ("transition tubercles") are not paired but are alternately placed. The cuticle has regular transverse rows of tiny dots, which are coarser between the rows of warts. The new species is saprobic and not, like *B. poligraphi*, associated with bark beetles. This is the first record of the subgenus *Rhodolaimus* in USSR.

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CONTROL OF PLANT PARASITIC NEMATODES

1. *Susceptibility of potato root eelworms and seed potato tubers to the vapour of sulphur dioxide*

BY

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These experiments were undertaken as part of a scheme for controlling the potato root eelworm, *Heterodera rostochiensis* Woll., in consignments of seed potatoes, where viable cysts may be found either in the soil adhering to the tubers or developing on the tubers themselves. As previous experiments had shown (Hague 1958) that the concentration-time product of methyl bromide required to kill the eelworm was in excess of that just not phytotoxic to the seed potato, it was decided to retest sulphur dioxide, a fumigant previously shown to be useful by Fenwick (1942).

Fenwick reported that sulphur dioxide was highly toxic to the potato root eelworm but caused little damage to seed potatoes. However, fumigation was done at high relative humidities and the cysts were thoroughly wetted before treatment.

In the following experiments, eelworm cysts and seed tubers were fumigated in the air-dried state, an environmental condition similar to that in which seed tubers are stored prior to transhipment. Eelworm cysts were also fumigated in a thoroughly moistened condition.

METHODS

In Fenwick's experiments the dosage of sulphur dioxide applied to the nematodes was not accurately measured. Therefore, in the absence of previous experience, the writer subjected the eelworm cysts and seed tubers to a range of sulphur dioxide concentrations. Fumigations were carried out in an air-tight cylindrical steel chamber of about 345 litres capacity and the sulphur dioxide was introduced by means of a specially constructed apparatus for measuring and vaporizing the fumigant. A description of the apparatus is given by Lubatti & Smith (1948). The

free-air space concentration was determined by withdrawing samples in evacuated vessels of known volume, the sulphur dioxide held in the vessel being determined as described by Lubatti (1950). The dosage of fumigant applied was measured as the concentration-time product. The concentration-time product is defined as the average concentration in the chamber multiplied by the duration of fumigation. The average concentration is determined by dividing the area under the concentration-time curve by the time of exposure to the fumigant. The sulphur dioxide concentration-time products are expressed as mg/l.

THE FUMIGATION OF FREE CYSTS OF THE POTATO ROOT ELWORM WITH SULPHUR DIOXIDE

Two populations of cysts (A & C) containing 211 eggs/cyst and 108 eggs/cyst respectively were fumigated in solid watch glasses, replication being threefold. Cysts, both air-dry and presoaked for one week were fumigated in batches of fifty. After fumigation they were assayed by the root-diffusate hatching technique (Perry & Beemster 1949).

The percentage mortality was estimated from the following equation:

$$\% \text{ Mortality} = \frac{L_U - L_F}{L_U} \times 100$$

where L_U is the mean number of larvae emerging from 50 cysts in the untreated controls and L_F is the mean number of larvae emerging from 50 cysts in the treatments. The concentration-time products to which the nematodes were subjected were estimated as above. Where cysts alone (i.e. without soil or tubers) were fumigated no appreciable loss of fumigant was detected and therefore the dosage applied was taken as the effective concentration-time product.

The assay results for air-dry cysts are shown in Table I. The encysted larvae in population A are evidently more susceptible to sulphur dioxide than those in population C. No larvae emerged from the presoaked cysts fumigated in any of the concentrations used. Thus, wet cysts are more susceptible to sulphur dioxide than air-dried cysts.

THE FUMIGATION OF SEED POTATO TUBERS WITH SULPHUR DIOXIDE

Eight commercial varieties of seed potato were fumigated for twenty hours at a series of concentrations. Gas samples taken from the free-air space in the chamber during the experiment showed that there was

TABLE I

The effect of fumigating air-dry cysts of H. rostochiensis *with sulphur dioxide*

C.T.P. mg.h./l.	*A cysts.* Emergence Total Larvae per 50 cysts	*A cysts.* % Mortality	*C cysts.* Emergence Total Larvae per 50 cysts	*C cysts.* % Mortality
84	60	99.5	3027	78.1
128	15		1840	86.7
192	12		1165	91.6
288	5	> 99.9	1280	90.8
432	0		2	99.98
648	5		0	100.0
Untreated	12590		13840	

a continuous loss of sulphur dioxide throughout the experiment, the fall in the free-air space concentration-time product being compared with the nominal concentration-time product in Table II. Because of this continuous loss of sulphur dioxide it was impossible to estimate the concentration-time product to which the tubers were exposed, and there-

TABLE II

Effect on the concentration-time product of sorption of sulphur dioxide by air-dry seed potatoes

Nominal Concentration-time products mg.h./l.	80	120	180	270	400
Free-air space. Concentration-time products mg.h./l.	22.5	45	79	116	158

fore the effective dosage was taken to be the average free-air space concentration-time product surrounding the tubers. The results are expressed in Table III as the proportion of tubers destroyed by the treatment.

DISCUSSION

For the fumigation of soil-free cysts, the above experiments confirm Fenwick's findings, wet cysts being more susceptible to sulphur dioxide than air-dry cysts. The fumigation of air-dry seed tubers shows that

TABLE III

The effect of fumigating air-dry seed potatoes with sulphur dioxide

Variety of Seed Potato	Number of tubers per treatment	Tubers destroyed % Concentration-time products mg.h./l. 22.5	45	79	116	158	Control
Majestic	25	76	80	96	100	100	4
King Edward	30	73	90	100	100	100	23
Great Scott	36	69	100	100	100	100	0
Arran Banner	29	59	100	100	100	100	0
Home Guard	35	86	100	97	100	100	11
Epicure	35	57	91	94	71	83	0
Arran Pilot	30	97	100	100	100	100	30
Sharpes Express	30	97	100	100	100	100	27

sulphur dioxide is very phytotoxic at low concentrations and the loss of sulphur dioxide during fumigation can probably be accounted for by solution in the water of the seed potatoes.

In Fenwick's experiments, the water sprayed onto the tubers may have wetted the adhering cysts and made them more susceptible and, at the same time, have acted as a barrier to the toxic action of the sulphur dioxide on the outer surface of the tubers. This may explain why no lasting damage occurred to the seed tubers while at the same time the adhering cysts were effectively killed. In the presence of free water, sulphur dioxide, because of its high solubility, would have to be continually supplied throughout the fumigation to be effective and for this reason it would probably not be so useful as the more sparingly soluble halogenated hydrocarbon fumigants. Methyl bromide (HAGUE 1958 and HARRISON & MAI 1956) and D-D and chlorobromopropane (VAN DER BRANDE, KIPS & D'HERDE 1954) have been shown to be more effective against moistened cysts. Furthermore methyl bromide is non-phytotoxic to seed tubers at 200 mg.h./l. (D. E. GRIFFIN in litt.) at which concentration-time product an effective kill may be obtained against moistened cysts (HAGUE 1958).

It is concluded that the fumigation of seed tubers and potato root eelworm cysts in the air-dry state is not a practicable proposition and that effective control will only be obtained by pretreatment of the cysts with water before fumigation.

Acknowledgment. This work has been carried out with the aid of a grant from the Agricultural Research Council whose assistance and interest are hereby acknowledged. The work forms part of a thesis approved for the degree of Ph. D. in the University of London.

ZUSAMMENFASSUNG

Bekämpfung pflanzenparasitärer Nematoden. I. Empfindlichkeit von Kartoffelnematoden und Saatkartoffelknollen gegenüber Schwefeldioxyd-dämpfen.

Die Bekämpfung von Cysten des Kartoffelnematoden *Heterodera rostochiensis*, die den Saatkartoffeln anhaften, ist ein wichtiges Quarantäneproblem. Lufttrockene Cysten und Saatkartoffeln sowie feuchte Cysten wurden in einer geschlossenen Stahlkammer mit Schwefeldioxyd begast. Diesen Stoff verwendete bereits FENWICK (1942). Die gasgeschwängerte Luft wurde in Proben der Stahlkammer entnommen, um die genaue Konzentration zu bestimmen, der die Kartoffeln und Cysten ausgesetzt worden waren. Die Mengenmessung erfolgte durch Ermittlung des Konzentration-Zeitproduktes in mg/Std pro Liter.

Die Lebensfähigkeit der begasten encystierten Larven wurde festgestellt, indem man sie in einem Kartoffelwurzeldiffusat zum Ausschlüpfen brachte. Die Ergebnisse stimmen mit denen von FENWICK überein: feuchte Cysten sind empfindlicher für SO_2 als trockene Cysten. Trockene Saatkartoffeln werden durch niedrige SO_2-Konzentrationen geschädigt. Das Absinken der SO_2-Konzentration ist wahrscheinlich auf seine Löslichkeit im Wassergehalt der Kartoffeln zurückzuführen.

Aus dieser Arbeit folgt, dass man eine wirksame Bekämpfung nur durch Begasen feuchter Cysten erreicht. Wegen des hohen Löslichkeitsgrades von SO_2 sind wahrscheinlich weniger lösliche Halogenkohlenwasserstoffverbindungen geeigneter.

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CONTROL OF PLANT PARASITIC NEMATODES

2. *Some aspects of the fumigation of the potato root eelworm,* Heterodera rostochiensis *Woll. with methyl bromide*

BY

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HAGUE (1959) has shown that methyl bromide does not control the potato root eelworm, *H. rostochiensis,* in the air-dry state, an environmental condition to which eelworm cysts would be subjected in consignments of seed potatoes. The present paper describes a series of methyl bromide experiments, the results of which may be important in the control of the potato root eelworm.

METHODS

Encysted potato root eelworms were fumigated with methyl bromide in an air-tight steel chamber, the concentration of fumigant to which the cysts were exposed being estimated by sampling the fumigant-laden air during the experiment. The methyl bromide in the sampling tubes was determined by the catalytic decomposition method of LUBATTI & BLACKITH (1956). The average concentration of methyl bromide in the free-air space of the chamber was multiplied by the duration of exposure to give an estimate of the concentration-time product of methyl bromide to which the eelworms had been exposed.

The unit of measurement was milligram hours per litre (mg.h./l.) which is equivalent for practical purposes to ounce hours per thousand cubic feet. (oz. hrs./1000 cu. ft.).

Soil-free cysts in the air-dry state were fumigated in batches of 50 or 100 in solid watch glasses. Viability after treatment was assessed by hatching the larvae in potato root diffusate (PETERS & FENWICK 1949) for three weeks, the cumulative hatch being expressed as the mean number of larvae emerging from the replicated batches of 50 or 100 cysts.

In the first two experiments described below the encysted eelworms were subjected to graded concentrations of the fumigant. Five dosage rates were chosen to give kills spread over a reasonably wide range.

The dosage-response curves obtained were analysed by regression analysis which has the advantage that the efficacy of treatments applied to the encysted larvae may easily be compared; the slopes, positions, and the L.D. 50's of the regression lines were the parameters investigated.

The percentage mortality was calculated from the emergence at each dosage by the following equation.

$$\% \text{ Mortality} = \frac{(L)_C - (L)_T}{(L)_C} \times 100$$

where $(L)_C$ is the mean number of larvae emerging from the replicated batches of cysts in the untreated controls and $(L)_T$ is the mean number of larvae emerging from the replicated batches of cysts in the treatments.

The percentage mortalities were transformed to the angular scale and plotted against the logarithm of the concentration-time product applied, which gives the best linearizing transformation for the particular set of data under study.

THE RESPONSE OF POTATO ROOT EELWORMS FROM DIFFERENT ORIGINS TO FUMIGATION WITH METHYL BROMIDE

Since it had been shown (HAGUE 1959) that cysts with the lowest contents were the most susceptible to methyl bromide, it was decided to find out whether this relationship was true over a range of concentrations.

Batches of 100 cysts from a natural infestation containing about 150 viable eggs per cyst and batches of 50 cysts from a pot-raised population containing about 420 viable eggs per cyst were fumigated at a series of concentration-time products, replication being threefold. The size grade of the cysts lay between 599 μ and 422 μ. The root diffusate assay was carried out immediately after the fumigation was completed, the number of larvae hatched in three weeks in the untreated controls being shown together with the results of the assay in Fig. 1. The natural infestation of the potato root eelworm was much more susceptible ($P < 0.001$) to methyl bromide than the pot-raised one, the L.D. 50's for the two populations being 44 mg.h./l. and 245 mg.h./l. respectively, thus confirming, over a range of doses, the results of previous work (HAGUE 1959). A stimulant action of methyl bromide at low doses was demonstrated in the case of the pot-raised cysts, the larval emergence at 44 mg.h./l. being greater than in the controls.

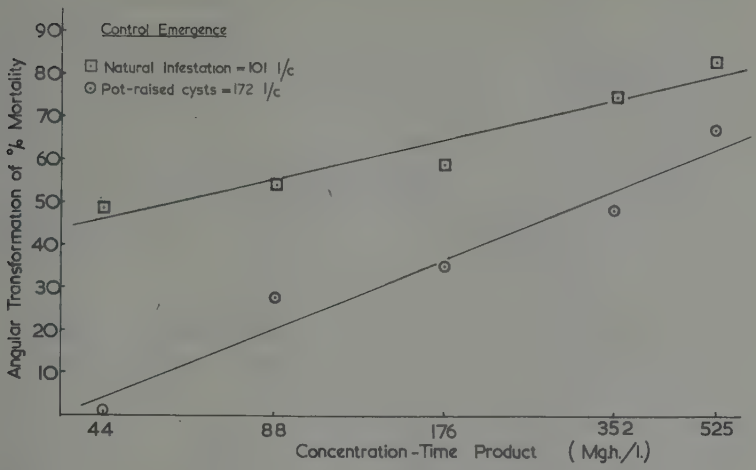

Fig. 1. The response of cysts of two populations of potato root eelworms to fumigation with methyl bromide.

THE RESPONSE OF POTATO ROOT EELWORMS FUMIGATED WITH METHYL BROMIDE IN SPRING AND AUTUMN

Two pot-raised populations C & D of the size grade $> 599\ \mu$ containing about 640 eggs per cyst and 630 eggs per cyst respectively were fumigated in batches of 50 in solid watch glasses, at a series of concentration-time products, replication being threefold. The fumigations were carried out in April and October. The results of the three week root diffusate assay are shown in Table I.

Encysted larvae fumigated in the spring are evidently much more susceptible to methyl bromide than those fumigated in the autumn ($P = 0.01$). As well as showing a seasonal response, the D cysts show the stimulatory effect of methyl bromide at low doses more effectively than do the C cysts.

THE RELATIONSHIP BETWEEN THE EELWORM RESPONSE AND THE TIME AT WHICH THE ASSAY IS CARRIED OUT AFTER FUMIGATION

It was decided to investigate whether an immediate estimate of kill (as carried out in previous experiments in this series) was a true measure of nematicidal activity. A natural infestation containing 450 eggs per cyst and a pot-raised population containing 630 eggs per cyst were fumigated at 288 mg.h./l. (36 mg./l. $\times$ 8 hrs.), a concentration-time

TABLE I

The response of cysts of two pot-raised populations of the potato root eelworm fumigated with methyl bromide in the spring and autumn

	C. Cysts				*D. Cysts*			
	Spring		*Autumn*		*Spring*		*Autumn*	
Concentration-time product mg. h./l.	Emergence Total larvae Per 50 cysts	% Mortality	Emergence Total larvae Per 50 cysts	% Mortality	Emergence Total larvae Per 50 cysts	% Mortality	Emergence Total larvae Per 50 cysts	% Mortality
56	—	—	13275 *	—	—	—	20580 *	—
84	9670	25.1	12172	6.0	13425	17.5	19394 *	—
128	—	—	10695	17.4	—	—	16218 *	—
192	1073	86.8	5673	56.2	3200	80.4	15660 *	—
288	713	94.5	2880	77.7	2295	85.9	3860	73.7
432	0	100.0	2477	80.8	1375	91.5	3400	66.8
648	11	99.9	—	—	340	97.7	—	—
Untreated Controls	12917		12953		16275		14660	

*) hatch greater than in the untreated controls.

product thought, from the results of previous experiments, to produce 50% mortality.

One day after fumigation, three replicated batches of cysts from each of the two cyst populations were soaked for one week and then a three-week root diffusate assay was performed, after which the cumulative hatches were counted. The same process of soaking and hatching was done at intervals of 2, 4, 8, 16 and 32 days after fumigation. The results of the assay are shown in Table II. Clearly mortality increases with the period of time elapsing between fumigation and assay.

DISCUSSION

It has been shown that satisfactory dosage-response curves can be obtained in eelworm assays (Fig. 1) and it would therefore be possible to compare the nematicidal properties of some unknown substance (such as a halogenated hydrocarbon) against a standard chemical of similar composition, the dosage-response relationship of which is known. Such a method could be of general application to nematicides in the same way as it has been to insecticides.

TABLE II

The effect of assaying the contents of cysts of potato root eelworms at different times after fumigation with methyl bromide

	Emergence, larvae per cyst	
Days after Fumigation	Pot-raised Population	Natural Infestation
1	450	75
2	300	25
4	263	12
8	304	28
16	210	19
32	222	4

After fumigation with methyl bromide the larval emergence in a root diffusate assay has been shown to decrease as the time between treatment and assay is increased. Similar results have also been reported by FENWICK (1957) working on ethylene dibromide.

Such effects suggest that caution should be used in evaluating results where insufficient time elapses between treatment and assay. It is not possible to predict from the above results what period of time should elapse between treatment and assay but it would appear that the longer the interval the better. Thus it might be advantageous to use either an

assay based on the final eelworm density after the host crop has been grown in the treated soil, or an assay based on the larval invasion of seedlings of the host plant grown in the treated soil. Assays of larval invasion and of the final population are measures of the ability of larvae to penetrate and to develop after treatment. These are the real answers required by the experimenter. Such assays could be used for all species of *Heterodera,* including those to which the root diffusate assay is not applicable.

The increased susceptibility of eelworms fumigated in the spring as compared with those fumigated in the autumn has also been reported by Feldmesser, Fassuliotis & Spruyt (1951), which suggests that eelworms in the spring may be in a more active physiological state than in the autumn. It would therefore be advantageous to fumigate eelworms at this least resistant stage. At this stage, however, the plant material to which the eelworms are adhering may also be in the most susceptible state. This is true of seed potatoes (Lubatti & Blackith 1950), and probably true of the resting stages of some other plants. Clearly the differing seasonal resistance of nematodes and plants to fumigants must be taken into account when control measures are advocated.

Natural infestations of the potato root eelworm are apparently more susceptible to methyl bromide than are populations raised in pots. The mechanism underlying this difference in susceptibility may be associated with the contents of the cysts; pot-raised cysts are of one age group and are mostly new and full. These cysts contain more viable eggs than cysts of natural infestations which are of varying age groups and have been partially emptied.

Other factors affecting the penetration of the fumigant may be associated with the packing of the eggs within the cyst, the presence or absence of interstitial material between eggs and the age of the eggs, old eggs being less virile than those in newly formed cysts. Thus in experiments with nematicides it is desirable to use new full cysts as the eggs contained are more difficult to kill and the nematicide is thus made to work under the most unfavourable conditions.

Evidence from the above experiments suggests that low doses of methyl bromide enhance emergence. Stimulatory effects with methyl bromide have been reported from onion seed tests by Lubatti & Blackith (1956) and from groundnut experiments (Somade 1955). Such stimulations are a common feature associated with the action of many drugs which become toxic if the level of treatment is raised sufficiently.

Acknowledgement. This work has been financed by a grant from the Agricultural Research Council, whose interest is hereby acknowledged. The work forms part of a thesis approved for the degree of Ph. D. in the University of London.

RÉSUMÉ

Essais de lutte contre les nématodes phytoparasitaires. 2. Quelques aspects de la fumigation du nématode doré de la pomme de terre (Heterodera rostochiensis *Woll.*) *au moyen de bromure de méthyle.*

L'auteur examine les techniques de fumigation au bromure de méthyle et les méthodes d'essai dans la lutte contre le nématode doré de la pomme de terre (*Heterodera rostochiensis.*) La relation entre le dosage du nématicide et la mortalité de cet organisme est étudiée à l'aide de l'analyse de régression. On obtient cette relation en fonction de la transformation angulaire du pourcentage de mortalité et du logarithme du produit concentration-temps. La susceptibilité du nématode doré au bromure de méthyle varie selon l'origine des kystes. Les kystes dont le contenu en oeufs viables est le plus bas semblent être les plus susceptibles. La fumigation des kystes est plus efficace au printemps qu'en automne.

Dans les essais la mortalité déterminée par l'emploi de la sécrétion radiculaire augmente quand on prolonge l'intervalle entre fumigation et essai. Des résultats obtenus il ressort que l'évaluation des résultats doit se faire après un intervalle suffisamment long.

On a aussi constaté que l'application de faibles doses de bromure de méthyle stimule l'éclosion des larves.

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PLANT SPECIES ATTACKED BY ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN THE FEDERATION OF RHODESIA AND NYASALAND

BY

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SUPPLEMENTARY LIST No. 1 *

SECOND COLUMN

Figures not enclosed by brackets

0 = No galls visible to the naked eye.
1 = Only one or two small swellings present.
2 = Few swellings present.
3 = Swellings or galls readily found.
4 = Swellings or galls visible at a glance.
5 = Numerous galls or swellings present.
6 = Roots almost totally galled or covered with swellings.

Figures enclosed by brackets

(0) = No egg masses seen.
(1) = Only one or two egg masses present.
(2) = Few egg masses.
(3) = Egg masses readily found.
(4) = Egg masses visible at a glance.
(5) = Egg masses numerous.

THIRD COLUMN

A = Single specimen examined.
B = Two to five specimens examined.
C = Six to ten specimens examined.
D = Eleven to twenty specimens examined.
E = More than twenty specimens examined.

Numbers 1, 2, 3 etc. indicate the actual number of infested sites from which specimens were obtained.

acr. = *Meloidogyne incognita* var. *acrita*
ar. = *M. arenaria*
hap. = *M. hapla*
jav. = *M. javanica*

Scientific, common and varietal names of plants.	*Meloidogyne* spp. with response and host values.	No. of observations and sites.
Abutilon grandiflorum Don	jav. 2	A. 1
Acacia baileyana F. v. Muell., Bailey's wattle	ar. 1, jav. 4	ar. A. 1, jav. B 2
Acacia cultriformis A. Cunn., Knife Acacia	ar. 5	B. 1

* The first list is published in *Nematologica* **3** (1958) : 332-349.

Acacia podalyriifolia A. Cunn. ex G. Don., Pearl Acacia	jav. 3	B. 1
Acalypha wilkesiana Muell. Arg.	ar. 3	A. 1
Albizzia chinensis (Osbeck) Merr.	jav. 3 (1)	A. 1
Albizzia lebbeck (L.) Benth.	jav. 1 (0)	A. 1
Alocasia sp., Elephant's Ear	ar. 2, jav. 1	B. 1
Aloe chabaudii Schon.	ar. 1, jav. 4	A. 1
Aloysia triphylla (L. Herit.) Britt. Lemon Scented Verbena	ar. 5 (2)	A. 1
Amarantus hybridus L. spp. *cruentus* (L.) Thellg. var. *paniculatus* (L.) (Thellg.)	acr. 5	B. 1
Ammi majus L., Queen Anne's Lace	ar. 2, jav. 4	ar. B. 1, jav. A. 1
Anemone coronaria L., var. St. Brigid	jav.3	B. 1
Id., var. De Caen	jav.3	B. 1
Anemone hupehensis Lemoine	acr. 4 (1), hap. 4 (1)	A. 1
Antirrhinum majus L., Snapdragon	ar. 5	C. 2
Aquilegia vulgaris L., Columbine	acr. 3 (2)	A. 1
Beta vulgaris L., Beetroot, var. Crimson Globe	acr. 6 (3)	B. 1
Id., var. Eclipse	jav. 5 (2)	A. 1
Blumea gariepina DC.	jav. 4	A. 1
Bombax malabaricum DC., Red or Silk Cotton Tree	jav. 3	A. 1
Brassica nigra (L.) Koch., Black Mustard.	ar. 1	B. 1
Brassica oleracea L. var. *acephala*, Kale, v. Dwarf Green Curled	ar. 2, jav. 2	C. 1
Brassica oleracea L. var. *capitata* L., Cabbage, var. Drumhead	acr. 3 (1), ar. 4	A. 1
Bulbine sp.	jav. 3	C. 1
Bulbostylis sp.	jav. 4	A. 1
Calendula officinalis L., Marigold	hap. 4	A. 1
Canna spp.	jav. 4	C. 2
Capsicum frutescens L. var. *grossum* Bailey, Bell or Sweet pepper	acr. 4	E. 1
Chrysanthemum leucanthemum L. hybrid, Ox eye daisy	hap. 5	A. 1
Clerodendrum sp., pale blue	ar. 3 (1)	B. 1
Coleus blumei Benth.	ar. 3, jav. 5 (2)	ar. B. 1, jav. A. 1
Coleus, cultivated hybrid	acr. 3 ar. 5	acr. B. 1, ar. E. 2
Corchorus junodii (Schinz) N. E. Br.	jav. 3	B. 2
Corchorus trilocularis L.	jav. 4 (3)	A. 1
Coriandrum sativum L., Coriander	acr. 3	E. 1
Cucurbita moschata Duch., Squash	acr. 3	A. 1
Id., id., var. Gem	acr. 4	A. 1
Cynara scolymus L., Globe Artichoke	jav. 4	A. 1
Datura cornigera Hook., Moonflower	hap. 3	A. 1
Daucus carota L., Carrot, var. Nantes	jav. 2	A. 1
Id., var. oxheart	jav. 4	A. 1
Emilia coccinea (Sims) G. Don	ar. 2, hap. 2, jav. 2	B. 1
Fimbristylis sp.	jav. 4	B. 1

Heliotropium arborescens L., Heliotrope or Cherry Pie	ar. 5 (3)	B. 1
Heterocentron roseum A. Br. & Bouché	acr. 3 (1), ar. 3 (1)	A. 1
Hibiscus cunnabinus L., Kenaf, var. Formosa	jav. 6	B. 1
Hibiscus panduriformis Burm.	jav. 1	A. 1
Hibiscus schizopetalus Hook., Fairy Hibiscus	jav. 5 (2)	B. 1
Iboza sp., Ginger Plant	jav. 5	A. 1
Iresine herbstii Hook. f.	ar. 3	A. 1
Iris tingitana Boiss. & Reut., Dutch Iris	jav. 2	B. 1
Jatropha podagrica Hook., Guatemala Rhubarb	jav. 3	B. 1
Kalanchoe marmorata Bak.	ar. 4 (2)	B. 1
Kalanchoe prolifera K. Hamet	jav. 5 (0)	A. 1
Lycopersicon esculentum Mill., Tomato var. Southland Marvel	jav. 4	A. 1
Manihot utillissima Pohl., Cassava or tapioca	acr. 3	B. 1
Medicago sativa L., Lucerne or Alfalfa. var. Flandria	jav. 3	B. 1
Id., var. Chartainvilliers	jav. 5 (3)	B. 1
Id., var. Caliverde	jav. 5	B. 1
Melilotus alba Desr., white Sweet Clover	jav. 2	A. 1
Ocimum americanum L.	jav. 3	A. 1
Opuntia sp., Prickly Pear	jav. 4	B. 1
Ornithogalum sp.	jav. 4 (2)	B. 1
Otiophora scabra Zucc.	jav. 3	A. 1
Papaver nudicaule L. Iceland Poppy	ar. 3	C. 1
Papaver rhoeas L., Shirley Poppy	ar. 1	B. 1
Pelargonium domesticum Bailey, Ivy Geranium.	ar. 3	B. 1
Penstemon barbatus (Cav.) Nutt.	jav. 3 (1)	B. 1
Penstemon tubiflorus Nutt. edesc.	jav. 3	B. 1
Penstemon sp.	jav. 2 (1)	A. 1
Peristrophe bicalyculata Nees	jav. 6	A. 1
Petunia sp.	hap. 5	B. 2
Phyla nodiflora (L.) Greene, Daisy Lawn	jav. 4	E. 1
Physalis angulata L.	acr. 2 (1), jav. 3	A. 1
Pisum sativum L. Garden Pea, var. Clipper	ar. 2	B. 1
Plectranthus sp. Pink and variegated	ar. 4, jav. 2	B. 1
Portulaca sp.	jav. 2 (0)	A. 1
Primula malacoides Franch.	hap. 3, jav. 4 (2)	B. 1
Psidium guajava L., Guava	ar. 1	B. 1
Rhynchosia minima (L.) DC.	jav. 4	A. 1
Rubus idaeus L., European red and yellow Raspberry.	ar. 2	E. 1
Rubus sp., Strawberry or Australian Raspberry	ar. 1, jav. 2 (1)	E. 1

Rudbeckia sp.	hap. 5	A. 1
Saccharum officinarum L., Sugar cane, var. Barbados 34104	jav. 4	B. 1
Id., id., var. Coimbatore 421	jav. 3	B. 1
Saintpaulia ionantha Wendl., African Violet	jav. 3	B. 2
Salvia splendens Sello, Scarlet sage	ar. 3	B. 1
Sechium edule Sw., Choko	jav. 3	A. 1
Sida cordifolia L.	jav. 3	A. 1
Sinningia speciosa Benth. & Hook. Gloxinia	jav. 3	A. 1
Solanum macranthum Dun., Tree Potato	jav. 6	B. 1
Solanum mammosum L., Pig's Ear Plant	jav. 6	C. 3
Solanum seaforthianum Andr.	acr. 5, ar. 3	B. 1
Strelitzia reginae Banks in Ait.	jav. 3	B. 1
Streptosolen jamesonii Miers, Flowering Marmalade	ar. 5	B. 1
Tribulus terrestris L., Devil's Thorn	acr. 2	A. 1
Tropaeolum majus L. Creeping Nasturtium.	ar. 5 (3)	B. 1
Verbena peruviana (L.) Britt	hap. 3, jav. 3	A. 1
Vigna unguiculata (L.) Walp., Cowpea or Kaffir Bean	jav. 6	E. 1
Viola tricolor L. vars., Pansy	jav. 3	A. 1

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THE EMERGENCE OF LARVAE OF *HETERODERA ROSTOCHIENSIS* WOLL. FROM SINGLE CYSTS

BY

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Cysts of the potato root eelworm are very variable in size and egg content. When eggs in unhatched cysts of known age are stimulated with root diffusate the emergence of the larvae from the cysts is also very variable. This variability may arise from the pretreatment of the cysts, (Boyd 1943a, Boyd 1943b, Ellenby 1946a, 1955, Fenwick 1951, Ferris & Mai 1956) the influence of the host-potato variety, (Gemmel 1943, Ellenby 1946b) the time of the year, (Lownsbery 1951, Winslow 1956) variable oxygen tension, (Triffitt 1930) the physical (Bishop 1953) and chemical environment (Ellenby 1946a) of the cyst, as well as variability which is "bound up with the nature of the cyst as a more or less closed system" (Ellenby 1956).

Batches of cysts are frequently used in experiments on the control of potato-root eelworm, in the bioassay of potato-root diffusate and in investigations into potato-root eelworm biotypes, and any information that could be applied to decrease their variability might increase the accuracy of the experiments. With this in mind the hatching behaviour of 200 individual potato-root eelworm cysts of various sizes was investigated.

MATERIALS AND METHODS

The cysts used were all produced on potatoes grown in 1954, the cyst stock originated from a plot at Rothamsted. A large random sample (several hundred cysts) was taken and divided into four size fractions A, B, C and D by passing it through sieves of 30, 40 and 50 meshes/inch. Fifty cysts were picked out at random from each size grade and each cyst was placed in about 0.5 c.c. of water in a covered solid watch glass and left to soak for ten days at 24°C. After draining off the water, 0.5 c.c. of potato-root diffusate was added to each cyst and was changed at weekly intervals. At the same time, the larvae which had

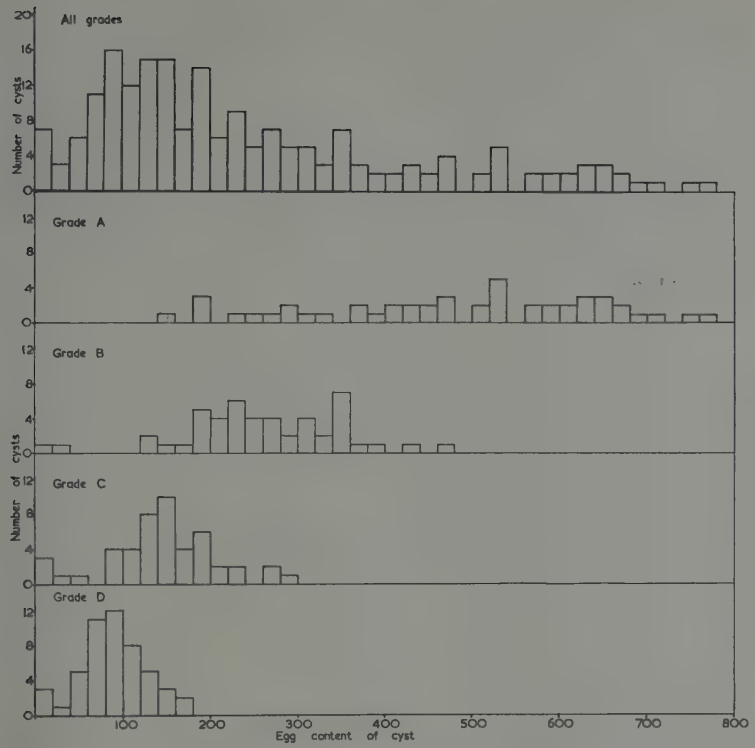

Fig. 1. Histograms showing the frequency distribution of *H. rostochiensis* cysts of similar egg content (class intervals of 20 eggs) based on 200 cysts from one source. The four lower histograms show the distribution for 50 cysts picked at random from four size grades (A, B, C, D; for sizes see Table 1 in text); the top histograms show the distribution for all the cysts.

hatched during the week were transferred to 2% formalin in a specimen tube. In this way all the larvae which hatched from each cyst were accumulated and preserved until hatching ceased at the end of five weeks. The number of hatched larvae per cyst was then counted and each cyst was opened and the remaining eggs also counted. From these figures the percentage hatch and the original egg content of each cyst was estimated.

RESULTS

The frequency distribution of the egg content of the cysts is shown in Fig. 1, and other data are summarised in Table I. The range of the

egg content of the largest cysts is wide. With decreasing cyst size the range of egg content decreases, and the histograms show peaks which are increasingly pronounced. In spite of the random selection of fifty cysts from each size grade, the distribution is skew (Fig. 1. "all grades"), and a large proportion of the cysts have a content of between sixty and two hundred eggs. Fig. 2 shows the frequency distribution of the percentage hatch per cyst, at class intervals of ten per cent. The frequency distribution of the percentage hatch for all cysts appears to be skew; more cysts in the three smaller grades exhibit a high percentage hatch than in the largest cyst grade.

TABLE I

Details of the size, egg content and "hatchability" of some cysts of Heterodera rostochiensis

	Cyst size mm	Cyst content range (eggs)	% hatch range	% mean hatch	Coefficient of variability of egg content. log. transf.	Coefficient of variability of percentage hatch. $\sphericalangle$ transf.
Grade A	$>$ 0.556	155-765	16-96	56.3	6.7%	26.7
„ B	0.556-0.401	125-465	50-94	78.9	9.8%	13.6
„ C	0.401-0.295	25-290	6-96	60.9	13.9%	28.8
„ D	$<$ 0.295	15-170	0-100	64.0	14.2%	24.6

variability of egg content of all cysts 16.6% (log. transf.)
variability of percentage hatching of all cysts 25.2% ($\sphericalangle$ transf.)

DISCUSSION

The results of this experiment are in agreement with ELLENBY (1946) who found that there was no linear relationship between the egg content and size of new cysts; there is no evidence, however, of two streams of hatching responses as shown by the same worker (ELLENBY 1956). Although the variability of the egg content of the cysts was reduced by sieving them into size grades, hatching variability was decreased only with the grade B cysts; over 65% of cysts of this size had a hatch of over 80%. The writer has found that field populations of *H. rostochiensis* are mainly composed (50%—60%) of cysts in the grade B size range so that selecting cysts of this size range for experiments entails less loss of the time and labour of cyst collection than when cysts of another size range are used.

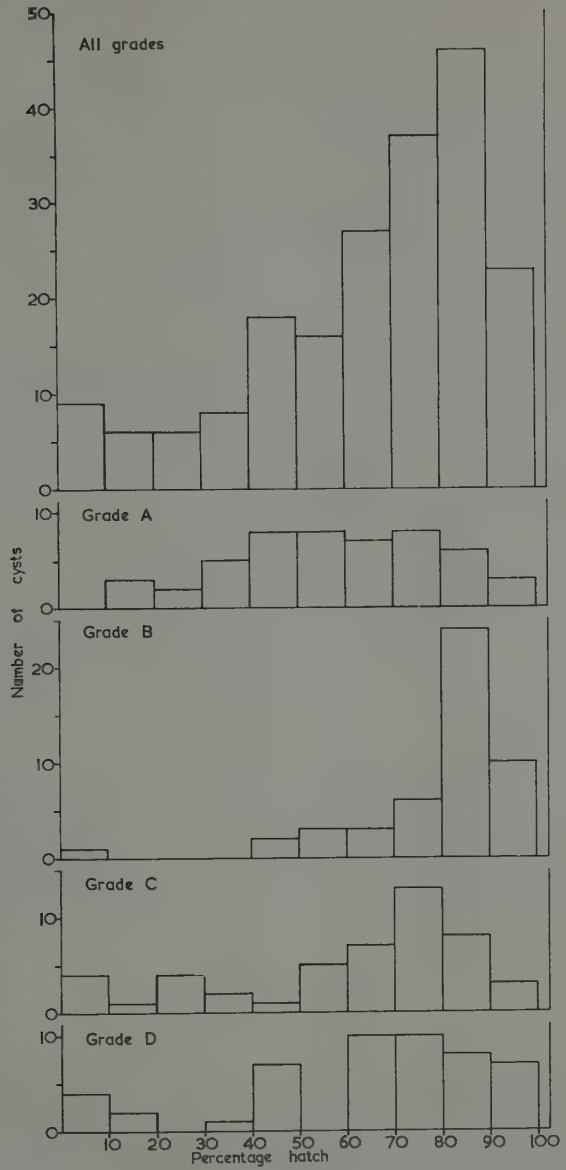

Fig. 2. Histograms showing the frequency distribution of the "hatchability" (expressed as percentage hatch) of 200 cysts from one source. The four lower histograms show the distribution for 50 cysts picked at random from four size grades (A, B, C, D; for sizes see Table 1 in text); the top histograms show the distribution for all the cysts. Class intervals of ten per cent were used.

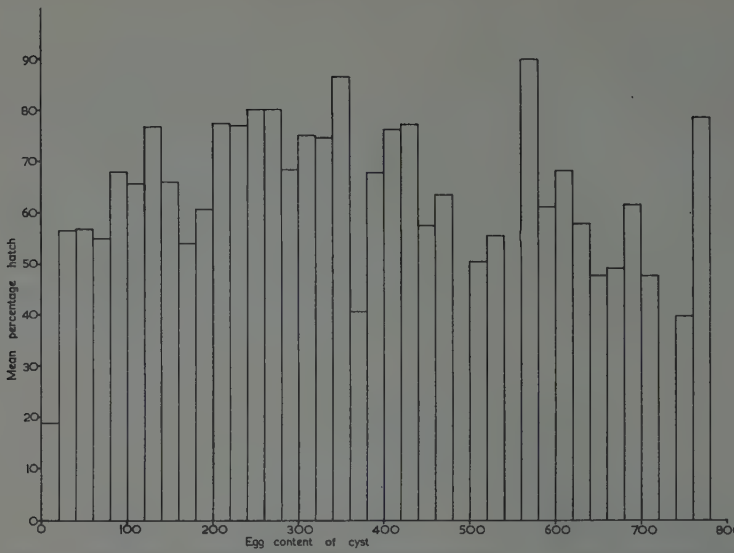

Fig. 3. Histograms showing the mean percentage hatch of cysts of similar egg content (class intervals of 20 eggs) and covering the content range of 200 cysts.

Ellenby (1946b) showed that a constant proportion of eggs hatched from cysts of one stock, and suggested (Ellenby 1946a) that the incomplete hatching from *H. rostochiensis* cysts is due to a "hatching inhibitor" produced by the larvae. Onions (1955) investigated the distribution of hatching within cysts of the potato root eelworm and suggested that hatching stopped due to oxygen deficit and increased acidity of the hatching medium within the cysts caused by the activity of the hatched larvae. From these hypotheses it might be expected that the percentage hatch would decrease with increase in egg content of the cyst. Some indication of this is shown by Fig. 3, and was noticed in the case of some cysts by Gemmel (1940).

ZUSAMMENFASSUNG

Das Larvenschlüpfen von Heterodera rostochiensis *aus Einzelcysten*

Frische Cysten von *Heterodera rostochiensis* wurden aus einer Herkunft beschafft und in 4 Grössenklassen eingeteilt. 50 aus jeder Gruppe zufällig entnommene Cysten wurden einzeln in Kartoffelwurzeldiffusat gelegt und die aus jeder Cyste geschlüpften Larven gezählt. Am Ende des Schlüpfprozesses erfolgte die Ermittlung der in jeder Cyste zurückgebliebenen Eier. Für jede Cystengrösse sind die Höhe der Eizahl sowie die Schlupfhöhe in Prozent und als Mittelwert in Tabelle I aufgeführt. Cysten von der Grössenordnung 0,556-0,401 mm scheinen den höchsten Schlupf zu haben; die Schwankungen aus diesen Cysten sind geringer als aus Cysten anderer Grössenklassen.

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SUSCEPTIBILITIES OF PLANTS TO THE CLOVER CYST NEMATODE, *HETERODERA TRIFOLII*, AND THE PERIOD REQUIRED TO COMPLETE A LIFE CYCLE [1])

BY

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The clover cyst nematode, *Heterodera trifolii* Goffart, 1932, which attacks several cultivated legumes, is fairly widespread in Canada (MULVEY, 1956). MULVEY (1958) showed that this nematode reproduces in the absence of males. The following is a report on the susceptibilities of some cultivated and weed plants to this nematode species and the number of generations produced in a year. All tests were conducted in a greenhouse at Ottawa.

MATERIALS AND METHODS

For the susceptibility studies sterilized soil artificially infested with large numbers (a minimum of one viable cyst per gram of soil) of the clover cyst nematode was used. The cysts were obtained from either mass or single cultures of *H. trifolii* on white Dutch clover grown in a greenhouse at Ottawa. Susceptibility was based on the degree of infestation of the roots by gravid white females.

For the studies of the number of generations, cultures from single larvae were obtained from white Dutch clover seedlings growing in 250-ml. beakers containing sterilized sand. Each plant was inoculated with an active second-stage *H. trifolii* larva by placing it close to the main root. A nutrient solution (Long-Ashton solution for clovers) was added to each beaker and the original level was maintained by daily addition of more solution.

Approximately 30 per cent of the larvae placed near the roots developed into gravid white females. Twenty-five single white Dutch clover seedlings growing in 5-inch pots containing sterilized soil compost were each inoculated with one of these gravid females and from

1) Contribution No. 3859, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

these pure line cultures were obtained. All subsequent inoculations were made with gravid females only. From gravid female to gravid female was considered a complete generation. In testing susceptibility the plants were allowed to grow at least for three months in the infested soil. These were then removed and the roots examined under the low power of the microscope.

SUSCEPTIBILITIES

TABLE I

Plants tested and degree of infestation by the clover-cyst nematode

Plant	Common name	No. of plants tested	Degree of infestation *	Culture
Leguminosae				
Trifolium repens L.	White Dutch clover	100	Very heavy	Single
Trifolium repens L.	Ladino clover	9	Light	Single
Trifolium pratense L.	Red clover	12	Nil	Single
Trifolium fragiferium L.	Strawberry clover	6	Light	Mass
Trifolium hybridum L.	Alsike clover	13	Nil	Mass
Trifolium incarnatum L.	Crimson clover	2	Nil	Mass
Trifolium medium L.	Zigzag clover	2	Nil	Mass
Pisum sativum L.	Garden pea			
	var. American Wonder	15	Very heavy	Single
	var. Lincoln	17	Very heavy	Single
	var. Laxton's Progress	9	Heavy	Single
	var. Topper	14	Medium	Single
	var. Early Wisconsin	18	Light	Single
Medicago sativum L.	Alfalfa			
	var. Rambler	2	Nil	Mass
	var. Ladak	2	Nil	Mass
Lotus corniculatus L.	Birds-foot trefoil	2	Nil	Mass
Glycine max Merr.	Soybean	12	Nil	Mass
Vicia villosa Roth.	Hairy vetch	15	Heavy	Mass
Phaseolus vulgaris L.	Garden bean			
	var Round Pod	4	Medium	Single
	var. Seminole	5	Light	Single
	var. Top Crop	5	Heavy	Single
	var. Tender Long	5	Very heavy	Single
Amaryllidaceae				
Allium cepa L.	Onion			
	var. Silver King	3	Nil	Single
	var. Red Globe	2	Nil	Single
	var. Giant Prize	7	Nil	Single
Cruciferae				
Brassica napobrassica Mill.	Rutabaga (Swede turnip)	5	Nil	Mass
Chenopodiaceae				
Beta vulgaris L.	Red beet	50	Nil	Single
Chenopodium glaucum L.	Oak-leaved goosefoot	20	Heavy	Single

* Nil, no nematodes; light, 1-5; medium, 6-25; heavy, 26-75; very heavy, 76 and over.

The above tests (Table I) show that the oak-leaved goosefoot *Chenopodium glaucum* was heavily attacked by *H. trifolii*. The generation was able to return to white Dutch clover. MULVEY (1957) found that the oak-leaved goosefoot was heavily attacked in the field by the sugar-beet nematode, *H. schachtii* Schmidt, 1871. Greenhouse tests with single cultures of *H. schachtii* showed similar results. The generation returned to red beet. Therefore, the oak-leaved goosefoot, which is widespread in Canada, is a common host of *H. schachtii* and *H. trifolii*.

Several varieties of peas, as shown in the tests, were heavily attacked by *H. trifolii*. The generation returned to white Dutch clover. FRANKLIN (1951) reported that larvae from cysts of *H. trifolii* would infect peas to a limited extent. WINSLOW (1954) found that garden pea was not attacked by *H. trifolii*. GOFFART (cited by FRANKLIN 1951) found that *Phaseolus vulgaris* became lightly infected whereas peas were not infected by *H. trifolii*. HASTINGS & BOSHER (1952) reported a species of *Heterodera* attacking peas in British Columbia.

The writer, as indicated, was unable after extensive tests to propagate the clover cyst nematode (from Canada) on red clover. FRANKLIN (1951) found that red clover was heavily attacked by *H. trifolii* in England. The writer, using cyst material from England, obtained similar results as those of FRANKLIN (1951) when this material was tested with red clover in a greenhouse in Ottawa.

GENERATION STUDIES

From February 15 to October 21, 1957, five generations were produced, or an average of 31 days for a generation. (Average soil temperature approximately 68 F.) Three generations were produced from December 10, 1957, to April 22, 1958, or an average of 45 days per generation. (Average soil temperature approximately 60 F.) Therefore, at least 9 generations of the clover cyst nematode may be produced in a year under greenhouse conditions. This species of *Heterodera* reproduces at any time during the year under favorable temperature and soil conditions.

Acknowledgements. The author is indebted to Dr. R. D. Winslow, Nematology Department, Rothamsted Experimental Station, England, for supplying some cysts, and to Mr. A. L. Sauve, greenhouseman, for his help in propagating and maintaining plants during the study.

ZUSAMMENFASSUNG

Die Empfindlichkeit verschiedener Gewächse gegenüber dem Kleecystennematode, Heterodera trifolii, *und die benötigte Zeit einen Lebenszyklus zu vollenden.*

Mehrere Sorten von Gartenerbsen (*Pisum sativum* L.) wurden von Kleecystennematoden (*Heterodera trifolii* Goffart, 1932) stark angegriffen, während Rotklee (*Trifolium pratense* L.) nicht befallen wurde. An meergrünem Gänsefuss (*Chenopodium glaucum* L.) traten Kleecystennematoden ebenfalls in starkem Masse auf. In Gewächshausversuchen mit Einzellarven konnten unter günstigen Boden- und Temperaturbedingungen während eines Jahres neun Generationen des Kleecystennematoden erhalten werden.

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R. H. Mulvey: Preliminary Studies on Oogenesis in a cyst-forming nematode, *Heterodera avenae* (Nematoda: Heteroderidae), *Nematologica* 4 (1959): 1-2.
Page 1, line 3 under Oocytes, read: "after" in stead of "and then".

Nematologica 4 (1959): 136-141. E. J. Brill, Leiden

TEMPERATURE AND ACTIVITY OF THE SUGAR-BEET NEMATODE AS RELATED TO SUGAR-BEET PRODUCTION

BY

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Early planting of sugar beets has been recommended for many years in California as a means of minimizing yield reductions by the sugar-beet nematode, *Heterodera schachtii* Schmidt. Lower winter temperatures are presumably more favorable for beet germination and growth than for nematode hatching, migration, and invasion of host roots.

Experimental evidence to substantiate this empirical recommendation is incomplete. BAUNACKE (1922) reported on temperature effects on the movement of sugar-beet nematode larvae and their rate of hatch from eggs in cysts. He found 16-28°C. (60.8-82.4°F.) to be the most favorable range for both movement and hatching, with hatching virtually absent below 10°C. (50°F.). NEBEL (1926), studying the invasion of *Brassica rapus* roots by free larvae at various temperatures, reported that infection did not occur at 6-9.5°C. (43.8-49.1°F.), proceeded slowly at 10-14°C. (50-57.2°F.), and was maximum at 18-20°C. (64.4-68°F.). However, 20°C. was the highest temperature tested. Nebel also studied the effect of temperature on the hatching of larvae from cysts. He found that total hatch at 30 days from 200 cysts ranged from 181, at 6°C. (43.8°F.), to 4158 at 25°C. (77°F.). Nebel found substantial hatching at the low temperatures of 6 and 9.5°C. (43.8 and 49.2°F.) which differs from Baunacke's findings.

DATE-OF-PLANTING FIELD TESTS

The importance of planting date to beet production in fields infested with sugar-beet nematode was first scientifically indicated in various soil fumigation tests conducted at Spreckels, Calif. (RASKI & LEAR, 1958). Generally, these tests showed increases in yields from fumigation. In plantings made between April 10 and May 20, untreated checks usually averaged 3.1-6.0 tons per acre [1]), and fumigated plots yielded as

1) 1 Ton = 901,2 kg; 1 acre = 0.4 hectare.

high as 13.8 tons per acre. In one test, however, the chemicals were applied in November and beets were planted in January. In this test, both treated and untreated plots produced an average yield of about 20 tons per acre.

These results were further investigated in date-of-planting tests conducted on the same field. Sugar beets were planted at monthly intervals in four double-row beds in each of four replicates randomized in four blocks. In every case but one, soil moisture was adequate at planting time or the rows were irrigated immediately after seeding. Yield data for two such tests, in 1955 and 1957, are shown in Table I.

Soil temperature records for a location about one mile east of the experimental plot were provided by the University of California Agricultural Extension Service at Salinas. These recordings, made throughout the year at a depth of six inches, include the maximum and minimum daily temperatures. The monthly averages during the 1955 and 1957 growing seasons are shown in Table I.

CONTROLLED TEMPERATURE TESTS

Two tests were carried out to determine more precisely the effect of temperature on the activity of sugar-beet nematode larvae. Temperature control was obtained by growing sugar beets under greenhouse conditions in containers that were submersed in water in constant-temperature tanks.

Test I. In the first test, sugar-beet seeds germinated 14 days previously in sand at normal greenhouse temperatures were transplanted to sterilized soil in 4″ clay pots. After 14 additional days the plants were removed from the pots and the entire soil and root ball placed in one-half gallon glazed crocks. Sterilized soil was added to fill the crocks to the desired level. This procedure was followed to permit selection of test plants for uniformity.

Sugar-beet nematode cysts were recovered from infested soil by wet screening through a 60-mesh screen. These cysts were transferred to small dishes of fresh water in which beet seedlings were placed to induce hatching of the larvae. Larvae were removed daily to ensure freshly hatched specimens. These were counted and added at the rate of 500 larvae per plant. Four holes about each test seedling were made to a depth of about 2″ into the root zone. A water suspension containing the 500 larvae was then introduced, putting about one fourth of the total volume in each hole. The holes were closed by scratching the sur-

TABLE I

Average sugar-beet yields in date-of-planting tests and average daily minimum and maximum soil temperatures at 6" depth

	1955			1957		
	Yield-tons per acre[a]	Ave. Min. Temp. °F.	Ave. Max. Temp. °F.	Yield-tons per acre[b]	Ave. Min. Temp. °F.	Ave. Max. Temp. °F.
Jan.	22.6	43	49	20.8[c]	43	50
Feb.	18.6	47	55	18.1	48	55
Mar.	8.8	51	62	8.8	51	59
Apr.	6.1	53	62	8.3	56	65
May		59	70		62	70
Jun.		67	77		68	78
Jul.		67	77		72	80
Aug.		68	77		70	78
Sept.		67	75		70	78
Oct.		63	71		—	—
LSD 19 : 1	5.1			4.2		
LSD 99 : 1	7.3			6.0		

[a] Harvest date — Oct. 27, 1955
[b] Harvest date — Oct. 2, 1957
[c] Planted Dec. 29, 1956 in dry soil and first rains occurred Jan. 11, 1957.

face of the soil and irrigating immediately. Five replicates were set up at each temperature, and five uninoculated plants were also included.

The crocks were then placed in the temperature-control tanks and the plants were allowed to grow for 50 days, during Dec., 1956, and Jan., 1957. Then the plant roots were washed free of soil as carefully as possible to avoid dislodging an excessive number of nematode cysts from the roots. The number of white females on the roots and damp-dry fresh weights of the plants are recorded in Table II.

TABLE II

Average fresh weights of plants and sugar-beet nematode counts in temperature control Test I

	Ave. plant weights in gms.		Ave. counts of white females arising from 500 larvae	
Temperature °F.	Check	Nematode added	Check	Nematode added
55	49.1	51.1	0	30.4
65	48.9	61.8	0	28.0
75	51.8	65.8	0	421.0
85	45.7	46.7	0	2.4
95	26.4	36.5	0	1.2
LSD 19 : 1	17.3	13.7		87
LSD 99 : 1	23.9	18.8		120

Test II. The second test, started in March of 1957, was similar to Test I except that several beet seeds were planted directly into the crocks. Subsequently the plants were selected for uniformity and thinned to one plant per crock. When the plants were about six weeks old, sugar-beet nematode larvae were introduced at two different levels, 500 and 5000. The plants were grown in the tanks for 53 days, in April and May, 1957, and then uprooted and washed free of soil. The numbers of white females were too great to be counted directly on the roots. Instead, the roots were placed on a 20-mesh screen held over a 100-mesh screen, and a strong stream of water dislodged the nematodes, which were caught on the finer screen. A careful examination of the washed roots showed this technique to be quite effective in separating the nematodes from the roots. Seldom did any white females remain on the roots. Lower numbers of nematodes were determined by direct count; higher numbers were computed from aliquots. Nematode counts and fresh weights are reported in Table III.

TABLE III

Average fresh weights of plants and sugar-beet nematode counts in temperature control Test II

	Average plant weights in grams			Average counts of white females		
Temperature °F.	Check	500 larvae	5000 larvae	Check	500 larvae	5000 larvae
65	105.1	107.2	99.9	0	17	401
70	105.7	91.7	91.1	0	145	4876
75	78.1	83.6	77.5	0	691	4231
80	79.6	78.4	85.9	0	971	5175
85	80.6	63.3	82.7	0	36	756
LSD 19 : 1	16.0	15.7	13.7	—	404	1526
LSD 99 : 1	21.7	21.2	18.5	—	548	2067

DISCUSSION

The nematode counts in Tests I and II are comparable, but may be as much as 50% below actual populations; later observations showed that the soil contains about as many females as are attached to the roots when they are washed. The two tests indicate that the optimum temperature range for sugar-beet nematode larvae is relatively narrow, 70-80°F. Activity was significantly lower at 65°F. and below, and at 85°F. and above. In Test II, where 500 larvae were introduced, the final population level at 70°F. was not as high as at 75 and 80°F. Where 5000 larvae were added, the resulting nematode population apparently

exceeded the maximum supportable by the plant size. In this case, plant size rather than temperature limited the population increase, and the final population at above 70°F. did not exceed the final population at 70°F.

Unfortunately, in the date-of-planting tests it was not possible to arrange harvest dates to allow each planting exactly the same number of growing days. However, past experience in this experimental plot has shown that beets heavily infested with sugar-beet nematode do not make continued satisfactory growth. It is believed that additional growing time for the later plantings would not have produced yields approaching those of the earlier plantings.

The soil temperature records show that an average maximum of 70°F. is not reached in that area until May; and that it was 77-78°F. during June. The yield records show that planting dates in January and February were the latest with which 18 tons per acre or more were obtained. With later dates the yields were reduced more than fifty percent. Since the temperature control tests indicate that 70°F. is near the minimum requirements for activity of sugar-beet nematode larvae, it appears that sugar beets may become well enough established and produce economical yields if sufficient growth can be made before soil temperatures reach this level. This does not show the maximum production that may be possible in such fields, since the nematode undoubtedly caused some reductions of yields in the early plantings.

ZUSAMMENFASSUNG

Temperatur und Aktivität der Rübennematoden in Verhältnis zum Zuckerrübenertrag

Zuckerrübenerträge von 18,1 bis 22,6 t je acre (= 0,4 ha) wurden auf einem mit *Heterodera schachtii* stark verseuchtem Feld in der Nähe von Salinas, Kalifornien, geerntet, wenn die Bestellung im Februar oder früher erfolgt. Eine Bestellung im März oder April brachte 6,1 bis 8,8 t je acre. Der Unterschied scheint auf die niedrigen Temperaturen in den ersten Monaten des Jahres zurückzuführen zu sein, die zwar das Keimen und das Wachstum der Rüben, jedoch nicht das Schlüpfen der Nematoden, ihre Wanderung und ihr Eindringen in die Wurzeln begünstigt. Versuche im Gewächshaus deuten an, dass die optimale Temperatur für die Aktivität der Nematoden 70 bis 80°F (= 21 bis 26,6°C) beträgt. Die Aktivität der Nematoden wurde bei 65°F und weniger (= 18,3°C) sowie bei 85°F und höher (= 29,4°C) stark vermindert. Feldbeobachtungen in Salinas zeigten, dass die durchschnittlichen Bodentemperaturen in 6 Zoll (= 15 cm) 70°F (= 21°C) vor Mai und Juni nicht erreichen. Dies deutet an, dass 2-3 Monate Rübenwachstum bei Bodentemperaturen unterhalb 70°F (= 21°C) auf nematodenverseuchtem Land deutlich ertragssteigernd wirken.

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DURCH DIE RÜBENRASSE DES STOCKÄLCHENS *DITYLENCHUS DIPSACI* HERVORGERUFENE SCHADBILDER BEI EINIGEN UNKRAUTARTEN

VON

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Nach Goodey (1941) treten an Rotklee und *Brassica napus*, die auf Böden wachsen, welche mit *Ditylenchus dipsaci* (Kühn) Filipjev aus Hafer verseucht sind, nur nekrotische Erscheinungen auf, während normale Befallsymptome ausbleiben. Nach Berichten von J. B. Goodey (1951) verursachen Älchen aus Rotklee Schwellungen an Blattstielen und Blattnerven von Erdbeeren. Bei Älchen anderer Herkünfte kräuseln sich die Blätter, obwohl in die Blattnerven keine Älchen eingewandert sind.

Neuerdings durchgeführte Untersuchungen von Seinhorst (1956) haben ergeben, dass die Reaktion der einzelnen Pflanzenarten auf eine bestimmte biologische Rasse des Stockälchens nicht immer gleich ist. Der Autor unterscheidet zwischen normalen und anomalen Befallsymptomen, die von den ersteren dadurch abweichen, dass :

1. nach Eindringen der Älchen eine leichte Nekrose oder eine Verkorkung der Zellen entsteht, die das befallene Gewebe umgeben.

2. gestauchter Wuchs und Deformationen an den Pflanzen auftreten, ohne dass Älchen eingewandert sind (Keine Auflösung der Mittellamelle).

3. stark gehemmter Wuchs, unregelmässige Verdickungen der Stengel und Blattstiele, sowie Missbildungen an den Blättern auftreten, nachdem nur einige Älchen in die Pflanzen eingewandert sind.

So zeigen Luzerne, Rotklee und Weissklee anomale Symptome, wenn sie von Älchen der Roggen-, Zwiebel- und Kartoffelrasse befallen werden. Es bildet sich nekrotisches Gewebe. Dieselbe Reaktion wird hervorgerufen, wenn stockälchenresistenter Rotklee mit der Rotkleerasse beimpft wird. Wird Flachs von den drei obengenannten Rassen befal-

1) Heutige Adresse : Services Agricoles, Luxembourg.

len, so zeigen die Pflanzen nachher gedrungenen Wuchs, ohne dass Älchen eingewandert sind. Dieselbe Reaktion zeigt *Euphorbia helioscopia,* wenn sie von der Roggenrasse befallen wird. Die Roggenrasse ruft ebenfalls an Lupinen und Bohnen anomale Befallsymptome hervor.

In den Kreisen Tauberbischofsheim und Donaueschingen (Baden-Württemberg) wurde in den Jahren 1954-1957 der Wirtspflanzenkreis der dort vorkommenden Rübenrasse des Stockälchens erfasst (Salentiny, 1957). Neben den hauptsächlichsten dort angebauten Kulturarten wurden auch die auf den verseuchten Feldern vorkommenden Unkräutern auf Befallsymptome untersucht. Die einzelnen Unkrautarten zeigen folgende Befallsbilder:

Ackergänsedistel (*Sonchus arvensis* L.), Abb. 1.
Windenknöterich (*Polygonum convolvulus* L.), Abb. 2.

Bei beiden Unkrautarten sind Sprossbasis und Blätter im Jugendstadium der Pflanzen stark angeschwollen. Die Blätter sind infolge Verdickungen der Mittelrippen, sowie des Blattgewebes stark verbogen und eingerollt. Die befallenen Pflanzenteile gehen rasch in Fäulnis über.

Ackergauchheil (*Anagallis arvensis* L.), Abb. 3.
Ackerhellerkraut (*Thlaspi arvense* L.).
Ackersenf (*Sinapis arvensis* L.). Abb. 4.

Im Jugendstadium sind Hypokotyl und Keimblätter angeschwollen, sodass ein Teil der befallenen Pflanzen abstirbt, Im späteren Entwicklungsstadium sind alle drei Unkrautarten, die den Jugendbefall überlebt haben, gestaucht und verkrümmt. Aufgeplatztes Gewebe, sowie Faulstellen mit Älchenbesatz wurden an kranken Pflanzen zu diesem Zeitpunkt nur mehr vereinzelt und zwar hauptsächlich in den Mittelrippen der Blätter beobachtet.

Ackerhahnenfuss (*Ranunculus arvensis* L.)
Klatschmohn (*Papaver rhoeas* L.)

Diese Pflanzen zeigten beim ersten Anblick äusserlich keine Befallsymptome. Bei näherer Betrachtung jedoch sieht man eine ganz leichte Anschwellung der Sprossbasis. Aufgeplatztes Gewebe sowie Faulstellen konnten niemals beobachtet werden. Auch an Blättern waren keine Befallsymptome vorhanden.

Ackerwinde (*Convolvulus arvensis* L.), Abb. 5.

Hier wurden im Keimblattstadium nur leichte Anschwellungen der

Keimblattstiele beobachtet. Sind die Pflanzen grösser geworden, so verkrümmten sich die Blattstiele, die Blätter rollten zusammen. In den meisten Fällen jedoch waren bei dieser Wildpflanze die typischen Befallssymptome (Verkrümmungen und Verbiegungen der Blätter und Stiele) vorhanden, ohne dass Älchen eingewandert waren.

Ausgebreitete Melde (*Atriplex patulum* L.).
Weisser Gänsefuss (*Chenopodium album* L.), Abb. 6, 7 u. 8.

Die Befallssymptome sind für diese beiden Pflanzen ähnlich. Am Hypokotyl treten im Keimblattstadium stecknadelkopfgrosse Verdickungen ähnlich wie bei Rüben auf, die nach einer gewissen Zeit aufplatzen (Abb. 6). Die Platzwunde, die sich der Rinde entlang nach oben und unten ausdehnt, bleibt meistens nur oberflächlich (Abb. 7). Manchmal waren die Älchen jedoch tiefer ins holzige Gewebe eingedrungen, das dann fein durchlöchert war (Abb. 8). An den Blättern selbst wurde niemals Befall beobachtet.

Bingelkraut (*Mercurialis annua* L.)

Beim Bingelkraut sind anfangs die Infektionssymptome ebenfalls ähnlich wie bei Rüben im Anfangsstadium des Befalls. In der Mitte oder am oberen Teil des Hypokotyls treten leichte Anschwellungen auf, die sich nachher vergrössern und aufplatzen. Im fortgeschrittenen Entwicklungsstadium der Pflanze trocknen diese Befallsstellen ein, sodass es niemals zu tiefgreifenden Zerstörungen kommt, jedoch zeigen die befallenen Pflanzen stark gestauchten Wuchs. Die Mittelrippen der Blätter sind ebenfalls angeschwollen und die Blätter verkrümmt.

Flohknöterich (*Polygonum persicaria* L.)

Im Keimblattstadium ist das Hypokotyl an seinen oberen Enden angeschwollen. Im fortgeschrittenen Entwicklungsstadium sind die Internodien verkürzt und die Triebe hauptsächlich im Bereich der Ochrea verdickt. Das befallene Gewebe wird braun und verfault.

Flughafer (*Avena fatua* L.)

Bereits einige Wochen nach der Keimung bilden sich beim Flughafer eine ganze Anzahl Schosser. Das Wurzelwerk ist kümmerlich ausgebildet, sodass bereits zu diesem Zeitpunkt viele Pflanzen absterben. Die zwiebelartige Verdickung am Stengelgrund ist stets vorhanden. Beim Ausgraben kranker Pflanzen bemerkt man Schosser mit bleichgelber Farbe, die nicht bis zur Erdoberfläche vordringen, sondern wieder in

TH. SALENTINY: *Schadbilder, hervorgerufen durch* Ditylenchus dipsaci.

Abb. 1. Verkrümmungen und Verdrehungen der Blätter, sowie Verdickung der Sprossbasis bei *Sonchus arvensis*.

Abb. 2. Anschwellung am oberen Ende des Hypokotyls bei *Polygonum convolvulus* (Links gesunde, rechts kranke Pflanze)

Abb. 3. Befall bei *Anagallis arvensis* (Ausgewachsene Pflanze)

TH. SALENTINY: *Schadbilder, hervorgerufen durch* Ditylenchus dipsaci.

Abb. 4. Gestauchter Wuchs bei einer ausgewachsenen Pflanze von *Sinapis arvensis*.

Abb. 5. Befall bei *Convolvulus arvensis*.

Abb. 6. Befall am oberen Ende des Hypokotyls bei *Chenopodium album*.

TH. SALENTINY: *Schadbilder, hervorgerufen durch* Ditylenchus dipsaci.

Abb. 7. Oberflächlicher Befall am Stengel bei *Chenopodium album.*

Abb. 8. Befall oberhalb der Wurzel bei *Chenopodium album.*

Abb. 9. Befall bei *Aethusa cynapium* im ausgewachsenen Stadium der Planze.

TH. SALENTINY: *Schadbilder, hervorgerufen durch* Ditylenchus dipsaci.

Abb. 10. Zwiebelartige Verdickung der Internodien bei *Galium aparine* (Links gesunde, rechts kranke Pflanze).

Abb. 11. Verdickung der Blattstiele bei *Lepidium draba* (Links gesunde, rechts kranke Pflanze)

Abb. 12. Befall bei *Stellaria media*. (Verkürzte Internodien und kleinere Blätter der befallenen Teile im Vergleich zu gesunden).

den Boden hineinwachsen. In den Monaten Juli-August beginnt bei den Pflanzen, die bis jetzt die Krankheit überstanden haben, das kranke Gewebe abzusterben. Meistenteils bleiben die Rispen in den Scheiden stecken. Älchenbesatz ist auch in den Halmknoten vorhanden.

Gemeines Kreuzkraut (*Senecio vulgaris* L.)
Gemeiner Hohlzahn (*Galeopsis tetrahit* L.)

Bei diesen beiden Unkrautpflanzen waren die Befallssymptome nur sehr leichter Natur, indem nur leichte Verdickungen der Blattmittelrippen zu Tage traten, die später aufplatzten. Am Hypokotyl wurden keine Befallssymptome beobachtet.

Grosser Wegerich (*Plantago major* L.)

Beim Grossen Wegerich, wo nur in einem Falle Stockälchenbefall festgestellt werden konnte, war die Sprossbasis leicht angeschwollen, während an den Blättern keine Befallssymptome vorhanden waren.

Hundspetersilie (*Aethusa cynapium* L.) Abb. 9.
Kleblabkraut (*Galium aparine* L.) Abb. 10.

Bei Kleblabkraut entsteht bereits im Keimblattstadium ein leichte Anschwellung am oberen Teil des Hypokotyls. Bei älteren Pflanzen sind die Internodien gestaucht und zwiebelartig verdickt. Bei Hundspetersilie wurde der Befall zuerst durch eine zwiebelartige Verdickung der Blattstiele sichtbar, die bei älteren Pflanzen auf die Sprossbasis übergreift. Bei beiden Unkrautarten geht das befallene Gewebe rasch in Fäulnis über. Bei Hundspetersilie greift der Befall schneller um sich und ist stärker ausgeprägt als bei Kleblabkraut. An den Blättern waren bei beiden Unkrautarten keine Befallssymptome sichtbar.

Nachtlichtnelke (*Melandrium noctiflorum* Fries.)

Bei der Nachtlichtnelke waren die Befallsymptome nur sehr leichter Natur. Die Sprossbasis war leicht angeschwollen, die Blätter leicht verbogen. Aufgeplatztes Gewebe konnte niemals beobachtet werden. Eine leichte, oberflächliche Faulstelle mit Älchenbesatz war nur in einem Falle vorhanden.

Pfeilkresse (*Lepidum draba* L.)

Am oberen Teil des Hypokotyls entsteht im Keimblattstadium eine leichte Verdickung. Im fortgeschrittenen Entwicklungsstadium sind die

Blattstiele etwas verdickt und runzlig (Abb. 11). Kranke Blätter fühlen sich auch etwas dicker an als gesunde. Manchmal entsteht auch am oberen Teil des Hypokotyls eine leichte Nekrose, die jedoch nur oberflächlicher Natur ist.

VOGELKNÖTERICH (*Polygonum aviculare* L.)

Beim Vogelknöterich traten im Keimblattstadium Verdickungen an den Keimblattstielen zu Tage. Im fortgeschrittenen Entwicklungsstadium waren die Pflanzen gestaucht, Blattstiele und Blätter verdickt, und die befallenen Teile aufgeplatzt. Faulstellen, von nur sehr leichter Natur, waren nur in sehr wenigen Fällen vorhanden.

VOGELMIERE (*Stellaria media* Villars.)

Die Vogelmiere zeigt leichte Einrollungen der Blätter und eine Verkürzung der Internodien. Auch scheinen die Blätter befallener Pflanzenteile kleiner zu sein als von gesunden (Abb. 12). Aufgeplatztes Gewebe, sowie Faulstellen konnten niemals beobachtet werden.

SUMMARY

Illustrations of injury caused by the sugar beet race of the stem eelworm, Ditylenchus dipsaci (Kühn) *on certain weeds*

Observations were made on the injury and symptoms caused to various weeds by *Ditylenchus dipsaci* derived from sugar beet. The most marked symptoms, which included splitting of tissue, malformation of leaves and stems, and ultimate decay of attacked parts, were shown by *Aethusa cynapium* L., *Avena fatua* L., *Polygonum convolvulus* L., *P. persicaria* L. and *Sonchus arvensis* L. Considerable injury without noticeable disturbance of tissues was shown by *Anagallis arvensis* L., *Convolvulus arvensis* L., *Mercurialis annua* L., *Sinapis arvensis* L. and *Thlaspi arvense* L. The least damage was shown by *Galeopsis tetrahit* L., *Lepidium draba* L., *Melandrium noctuiflorum* Fries., *Papaver rhoeas* L., *Ranunculus arvensis* L., *Senecio vulgaris* L., and *Stellaria media* Villars, while *Atriplex patula* L., *Chenopodium album* L., *Galium aparine* L., *Plantago major* L. and *Polygonum aviculare* L., occupy an intermediate position.

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INVESTIGATIONS ON THE CLOVER CYST NEMATODE, *HETERODERA TRIFOLII* (NEMATODA: HETERODERIDAE) [1]

BY

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The clover cyst nematode, *Heterodera trifolii* Goffart, 1932, appears to be fairly widely distributed in Canada (Mulvey, 1956) and has been found on several species of Leguminosae as well as some weed hosts (Goodey, 1956; Mulvey, 1959). Reproduction is parthenogenetic so that males are unknown (Mulvey, 1958). Franklin (1951) and Hirschmann (1956) have studied the morphology of the second-stage larvae and the adult female, respectively. The morphology of the other larval stages has not been described and the life history has remained unrecorded. This report attempts to cover these gaps in our knowledge. Because of their taxonomic importance the development of the fenestralia is given particular attention. Terminology for these and other structures in the vaginal cone of the cyst follows Cooper (1955a), terminology for the parts of the spear follows Raski (1950).

MATERIALS AND METHODS

The method used by Raski (1950) in his study of the life history of the sugar-beet nematode, *H. schachtii* Schmidt, 1871, was followed except that white Dutch clover seedlings were used as a host plant.

During the period of development, soil temperatures were recorded daily. The temperatures ranged from 51-68° F, with an average of 60° F. The study was conducted from November 14, 1957, to January 16, 1958.

Permanent slide mounts of cones (the posterior part of the cyst) were prepared as described by Cooper (1955b).

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1) Contribution No. 3892, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

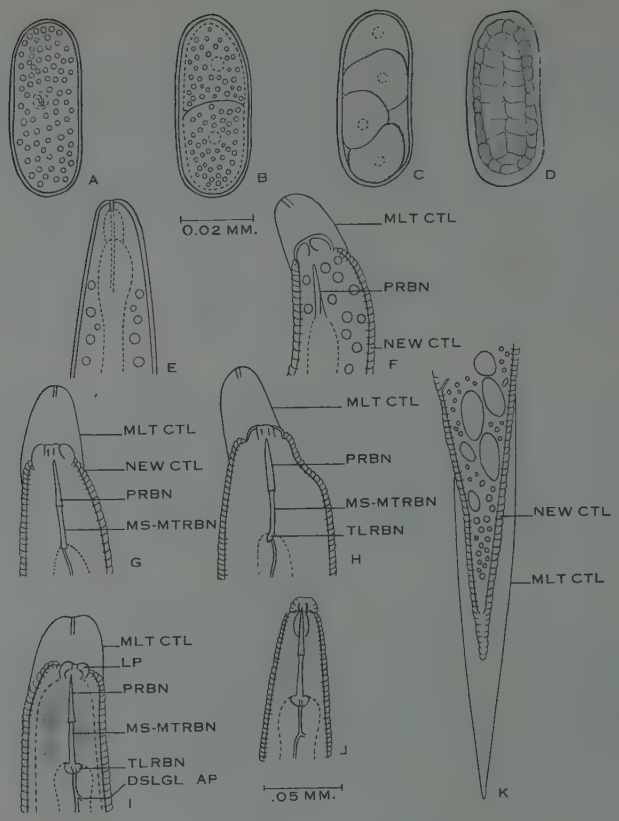


Fig. 1. A-D, Egg to embryo stages of *Heterodera trifolii*. E. Head view of first-stage larva; F-I, K, Molting of first-stage larva showing development of stylet and casting of cutcle. J, Head view of second-stage larva.
Legends to the abbreviations, used in the figures, are given at the end of this paper.

DESCRIPTIONS AND DEVELOPMENT OF LIFE STAGES

Egg

The mature eggs (Fig. 1, A) range from 100 to 110 μ in length and from 40 to 45 μ in width. First cleavage produces two equal cells within the egg wall (Fig. 1, B). Second cleavage (Fig. 1, C) is followed by rapid mitotic division. Eventually the egg is composed of a large number of cells (Fig. 1, D) in which two layers can be distinguished. Embryonic development follows closely that described for the sugar-beet

nematode by RASKI (1950). As the embryo increases in length it becomes more slender and eventually, through progressive increase in length, 2-3 flexures are formed within the egg shell.

First-stage Larva

There is little differentiation in the head region (Fig. 1, E) of the first-stage larva. The first molt occurs within the egg shell. The spear becomes visible shortly after the first molt begins. The prorhabdion region is formed first (Fig. 1, F), the meso-metarhabdion region (Fig. 1, G) next, and finally the telorhabdion (basal knobs) region (Fig. 1, H and I). In the fully developed second-stage larva (Fig. 1, J) the spear is fully developed. The lip region is set off by a constriction.

Second-stage Larva

The second-stage larva (Fig. 2, A) varies in length from 475 to 490 μ, and in width from 20 to 22 μ. The annular striation in the spear region measures 1.3 μ and at the middle of the body about 1.5 μ. The cephalic framework is heavily sclerotized. The heavy spear is about 24 μ long (Fig. 2, A). The genital primordium is situated slightly posterior to the middle of the body and is 15 μ long and 10 μ wide. The second-stage larva, after completion of its development, hatches from the egg and begins its search for a suitable host plant. The infective second-stage larvae were found in the main and lateral plant roots; it appears from the roots examined that entry is not confined to the root tip.

Second Molt

Evidence of the second molt was found 5-6 days after the larvae penetrated the roots. Molting appears to begin in the posterior part of the larva and shortly thereafter in the anterior region. In this stage the esophageal glands become shortened and the nucleus greatly enlarged. Within the cast skin the third-stage larva (Fig. 2, B) measures from 400 to 460 μ in length and from 30 to 50 μ in width. The genital primordium averages 40 μ in length and 20 μ in width and appears to undergo little change during this phase of development. The rectum is well developed. By the 12th day all the larvae examined had completed the second molt.

Third-stage Larva

The larva of this stage (Fig. 3, A) varies in length from 400 to 460

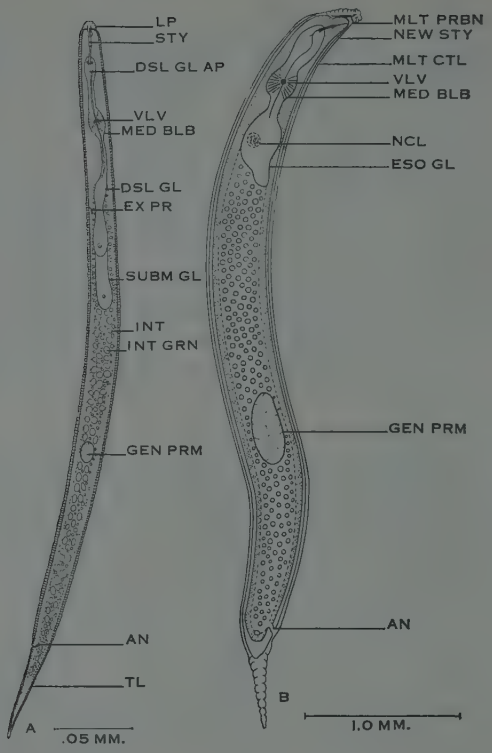


Fig. 2. A, Second-stage larva. B, Second-stage larva molting, showing shortened esophageal gland and casting of cuticle and stylet of second-stage larva; third-stage larva shown within the second-stage larval skin.

μ and in width from 53 to 96 μ. The head region has 7-8 annules and tapers almost to a point (Fig. 3, A). Annulations are absent in the rest of the body cuticle. The spear is 20-21 μ long and has fairly distinctive basal enlargements but is much less robust than that of the second-stage larva. The medium bulb is large and has a very conspicuous valve. The esophageal gland, which has shortened considerably, has a large nucleus. The reproductive system has begun development by the growth of two oval enlargements arising from the genital primordium. In this stage the ovaries undergo considerable development (Fig. 3, B). The third-stage larva reached full growth 13-15 days after the second-stage larva penetrated the host.

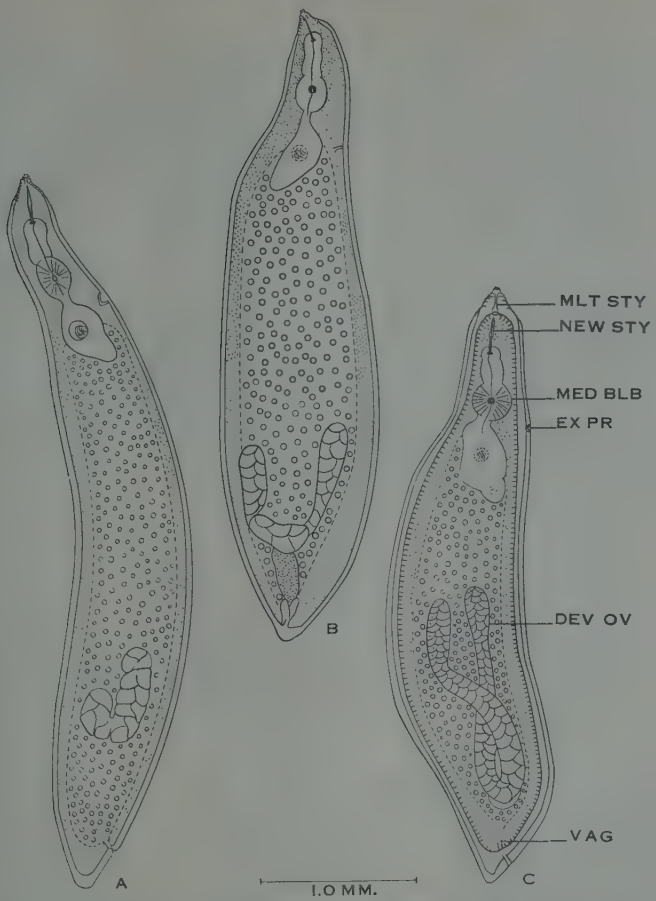


Fig. 3. A, Early third-stage larva showing beginning of ovarian development. B, Older third-stage larva showing ovaries more developed. C, Third-stage larva molting.

Third Molt

At this stage the reproductive system has increased rapidly in length (Fig. 3, C). The body has increased in width. Evidence of the third molt was observed on the 13th day after larval penetration of the plant root. By the 17th day most of the larvae had completed this molt.

Fourth-stage Larva

The fourth-stage female larva (Fig. 4, A) is flask-shaped and varies

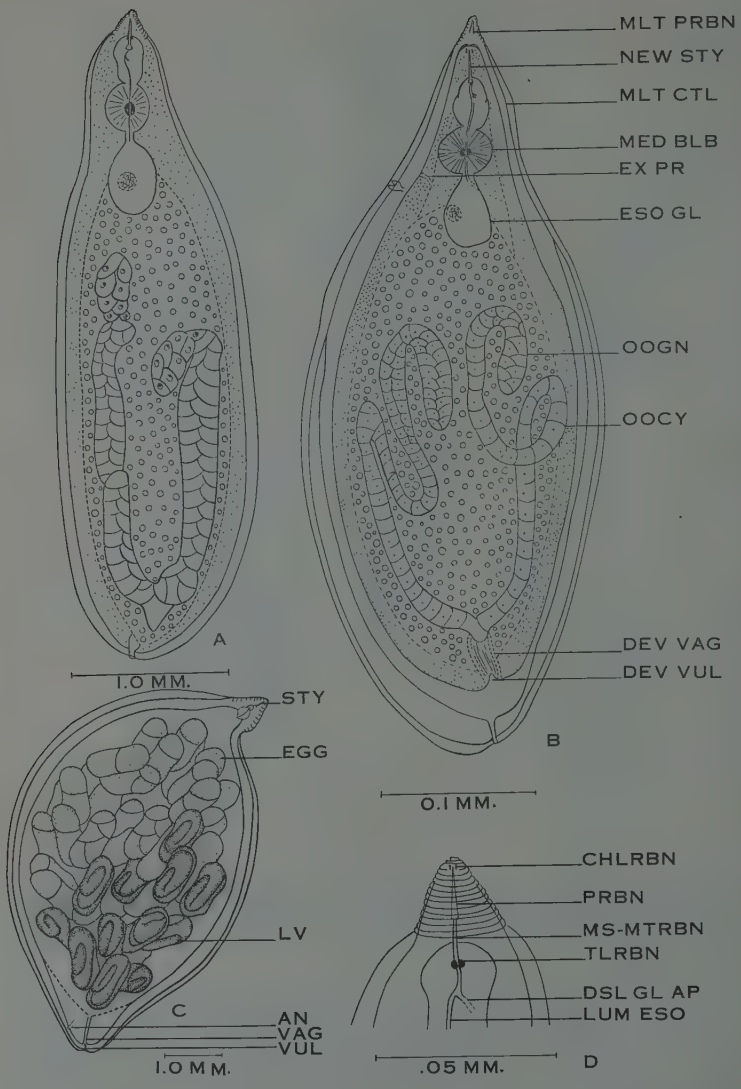


Fig. 4. A, Early fourth-stage larva showing flexure in ovaries. B, Older fourth-stage larve with reproductive organs well developed. C, Mature white female of *H. trifolii* showing eggs and larvae. D, Anterior area of fourth-stage larva.

from 410-500 μ in length to 125-160 μ in width. The body cavity is surrounded by a distinctive granular layer. The ovaries undergo considerable development during this stage and the cuticle is marked by irregular longitudinal lines. The spear is long and slender and has distinctive basal enlargements (Fig. 4, D). Development of the fourth-stage larva is completed 21 days after penetration of the root.

Fourth Molt

Evidence of the fourth molt was observed 22 days after penetration of the root. By the 26th day most of the larvae had completed this molt, the last. During this molt the reproductive system gains access to the exterior by formation of a terminal vulva (Fig. 4, B). Very shortly after the molt a gelatinous matrix is formed around the female posterior protuberance. This structure has an inner and an outer gelatinous, transparent layer.

Adult Female

Twenty-nine days after penetration, of the 29 adults examined four contained 1 egg each and two 3 eggs each. Forty-three days after penetration, several females were filled with eggs. Many of these eggs contained first-staged larvae (Fig. 4, C).

White Female to Brown Cyst Stage

Fifty-six days after penetration the first eggs containing second-stage larvae were found in the gelatinous matrix. Yellowing of female cuticle had also begun. By the 60th day many females had reached the yellow phase. Clover plants whose roots were heavily infested with females showed very poor growth, both above and below ground. The first brown cysts were found 64 days after the larvae had penetrated the clover roots.

DEVELOPMENT OF CONE TOP AND UNDERBRIDGE

The semi-fenestrae and underbridge (Fig. 5, A, B and C) in mature cysts of *H. trifolii* are generally well developed and different from those of some other cyst-forming species. The following is an account of the development of these structures:

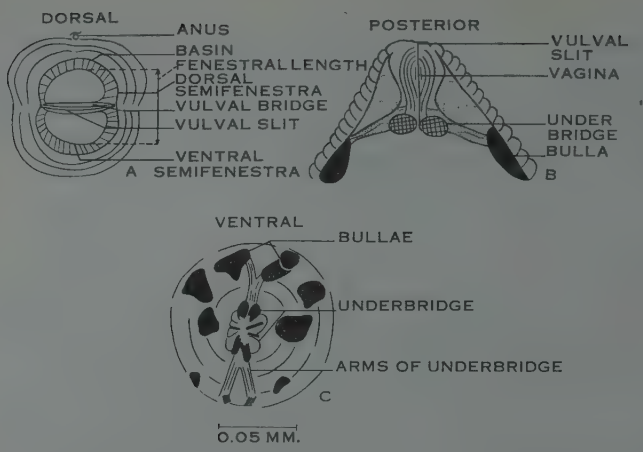


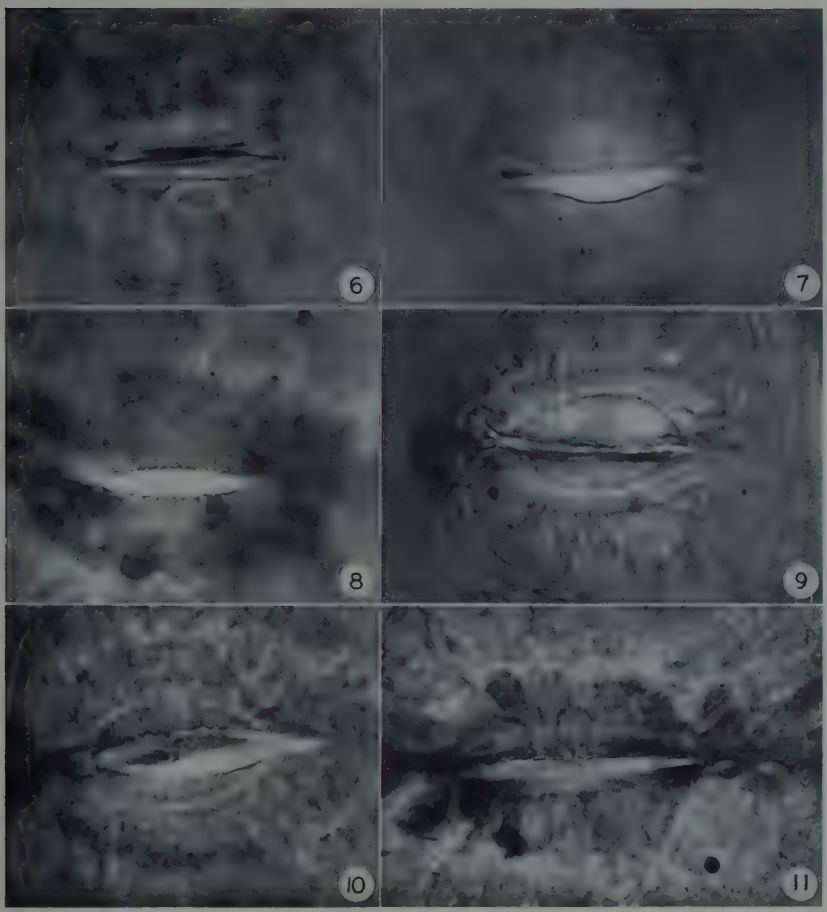
Fig. 5. Camera lucida drawings of fenestrated cone top of *Heterodera trifolii* cyst indicating positions of the structures studied. A, Posterior view of cone top. B, Side view (dorso-ventral aspect) of cone top and underbridge. C, Anterior view of underbridge.

White Female to Yellow Phase

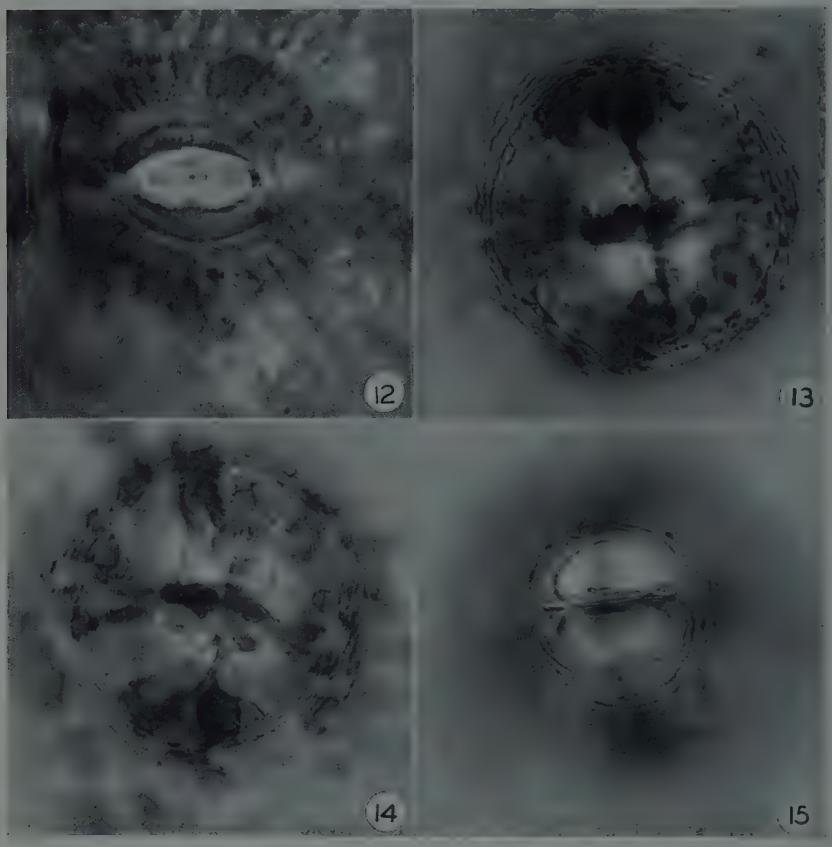
The vulva of the white female is generally open and bounded by thin parapets (Fig. 6). The semi-fenestral areas are faintly outlined. The vaginal lining is serrated. The vulva of a female which has reached the early yellow phase is generally open, the vulval bridge is thin, and there is a slight indication of the semi-fenestral contours (Fig. 7). The underbridge as yet shows little development. The vaginal lining is striated and the walls fairly thick (Fig. 8). In the mid yellow phase the semi-fenestrae appear transparent and the vulval bridge has thickened (Fig. 9). The vaginal lining is distinctly serrated and the walls are thick (Fig. 10). The underbridge is weakly developed at this stage. During the yellow-brownish stage the underbridge shows slight pigmentation (Fig. 11). As the underbridge develops it becomes more deeply pigmented and the bifurcate arms are distinct. The vaginal lining is serrated (Fig. 12) and the walls are thick.

Brown Cyst Stage

Newly formed brown cysts show an unnamed structure (Figs. 13, 14) just below the level of the fenestra (about 16-18 μ) and 24-27 μ deep. This structure runs at right angles to the underbridge and vulval bridge. It may be hardened muscle tissue that in the white female con-



Figs. 6-11. Photomicrographs showing cone top and underbridge structures of *H. trifolii*. 560x. 6, Gravid white female showing vulval opening and outlines of fenestra. 7, Yellow phase showing vulval opening, hyaline parapets, and beginning of fenestration. 8, Yellow phase showing vaginal walls and serrated vaginal lining. 9, Late yellow phase showing transparent fenestra, vulval slit, and vulval bridge. 10, Late yellow phase showing heavily striated vaginal lining and thick walls. 11, Yellow-brownish phase showing underbridge, vagina, beginning of formation of bullae, and bifurcation of the arms of underbridge.



Figs. 12-15. Photomicrographs showing cone top and underbridge structures of *H. trifolii*. 560x. 12, Early brown-cyst stage showing serrated vaginal lining and thick walls. 13, Newly formed brown cyst showing muscle structure at right angles to vulval bridge and photographed close to level of fenestra. 14, Same as 13, but photographed at top level of the underbridge, which is at right angles to the muscle structure. 15, Brown-cyst stage showing fenestra, vulval bridge, and basin.

trolled the opening or closing of the vagina. The underbridge is now fully developed (Fig. 14), pigmented, and fairly heavy, and has bifurcate arms. The semi-fenestrae (Fig. 15) are transparent and distinct and the vulval bridge is thin. The vulval slit is sometimes open. The basin is distinct.

DISCUSSION

Since *H. trifolii* is parthenogenetic this study was necessarily restricted to the female. The time taken for development was probably prolonged by the low soil temperature. Raski (1950) found that at an average soil temperature of 66.8° F the time required for development of the sugar-beet female was 17 days after larval penetration of the beet root and larvae were produced by the 31st day. The present investigation showed that development of the clover-cyst nematode at an average soil temperature of 60.0° F required 26 days for the female adult and 43 days for production of larvae. Ichinohe (1955) reported that in the soy-bean nematode, *H. glycines* Ichinohe, 1952, from the time of larval entry to the time of emergence of the embryonated eggs within the egg sac takes a minimum of 24 days at an average soil temperature of 74° F and a maximum of 41 days at 64° F. Temperature apparently has a pronounced effect on the rate of larval development in this species.

Acknowledgment. The author is indebted to Mr. A. L. Sauve, greenhouseman, for propagating and maintaining the plants during the study.

ZUSAMMENFASSUNG

Untersuchungen an dem Kleezystennematoden (Heterodera trifolii) (*Heteroderidae: Nematoda*)

Der Kleezystennematode, *Heterodera trifolii* Goffart, 1932 bildet fünf Stadien aus. Das erste Larvenstadium häutet sich innerhalb des Eies und entwickelt eine vollständig ausgebildete Larve II, die gegebenenfalls in die Pflanzenwurzel einwandern kann. Innerhalb der Pflanzenwurzel nimmt die Larve II drei Häutungen vor, bis sie das adulte Stadium erreicht. Bei einer durchnittlichen Bodentemperatur von 60°F (= 15,5°C) begann die zweite Häutung 5-6 Tage, nachdem Larve II in die Pflanzenwurzel eingedrungen war. Die meisten Larven vervollständigten die Häutung bis zum 12. Tage. Die dritte Häutung war am 17. Tag, die vierte und letzte Häutung am 26. Tag beendet. In dem erwachsenen Weibchen traten die ersten Eier am 29. Tag auf. 43 Tage nach dem Eindringen in die Wurzel waren die meisten Weibchen mit Eiern angefüllt. Viele Weibchen erreichten die Gelbphase am 60. Tag; die erste braune Zyste wurde am 64. Tag gefunden.

Die Vulvabrücke des Weibchens während der Gelbphase ist dünn, die Halbfenster sind schwach ausgebildet. Die Unterbrücke, die während der mittleren

Gelbphase schwach entwickelt ist, zeigt leichte Pigmentierung während der gelbbraunen Phase und wird tief pigmentiert in der neugebildeten braunen Zyste. Die zweigabeligen Äste der Unterbrücke sind deutlich erkennbar während des letzteren Stadiums. Neugebildete Zysten zeigen eine unbekannte Struktur gerade unter der Fensterhöhe; diese kann verhärtetes Muskelgewebe sein.

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Abbreviations. — AN, anus; CHLRBN, cheilorhabdion; CTL, cuticle; DEV OV, developing ovary; DSL GL, dorsal gland; DSL GL AP, dorsal gland aperture; ESO GL, esophageal gland; EX PR, excretory pore; GEN PRM, genital primordium; INT, intestine; INT GRN, intestinal granule; MED BLB, median bulb; MLT CTL, molting cuticle; MLT PRBN, molting prorhabdion; MS-MTRBN, meso-metarhabdion; NCL, nucleus; OOCY, oocyte; OOGN, oogonia; OV, ovary; PRBN, prorhabdion; STY, stylet (spear); SUBM GL, submedian gland; TL, tail; TLRBN, telorhabdion; VAG, vagina; VLV, valve; VUL, vulva.

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THE EXCRETORY SYSTEM OF *PARAPHELENCHUS* AND THE IDENTITY OF THE HEMIZONID

BY

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Dr. B. C. Chitwood (in litt.) has questioned the writer's interpretation of the excretory system of *Paraphelenchus* (GOODEY, J. B., 1958), and has also pointed out the probability that the hemizonid (GOODEY, J. B., 1951) is a ventro-lateral commissure of the nervous system. This short note records the results of further examination of these two points.

EXCRETORY SYSTEM OF PARAPHELENCHUS

In describing *P. myceliophthorus* the writer commented that the excretory system appeared to consist of a ventral cell joined by the excretory duct to the excretory pore. In specimens of this species taken from agar plates on which they are actively feeding, the only part of the excretory system to be seen, and then only with considerable difficulty, is that just mentioned. So that further details could be seen, specimens were kept in water in a refrigerator for three to four months, when the contents of the gut cells were almost used up and details of the interior structure of the nematodes became more visible. This showed that the above description is incomplete, though very many specimens had to be examined before the details could be seen clearly. Specimens were mounted in water and examined about 1½ hours after sealing the coverslip, by then the nematodes had usually turned onto their dorsal sides. On the specimens examined, the excretory duct was about 30 μ long before it joined the ventral cell. This duct was in two parts, that joining the excretory pore being about 20 μ long and very transparent, whereas the second, shorter, part was less transparent and very clearly visible. This second part of the duct, near its junction with the excretory cell, branches laterally, giving rise to an anterior and a posterior tubule which run across the body tissues into the right lateral chord (Fig. 1). Two specimens seemed to have two posterior tubules.

one passing to the right the other to the left lateral chord, but this could not be confirmed although many further specimens were examined.

While examining these specimens of *Paraphelenchus* I once observed the rapid expulsion from the excretory pore of a short length of some excretory product. This remained in the water near the animal retaining its stringlike form but swelling slightly.

HEMIZONID

In recent examinations of nematodes Dr. Chitwood's suggestion about the identity of the hemizonid has always been in my mind. I have

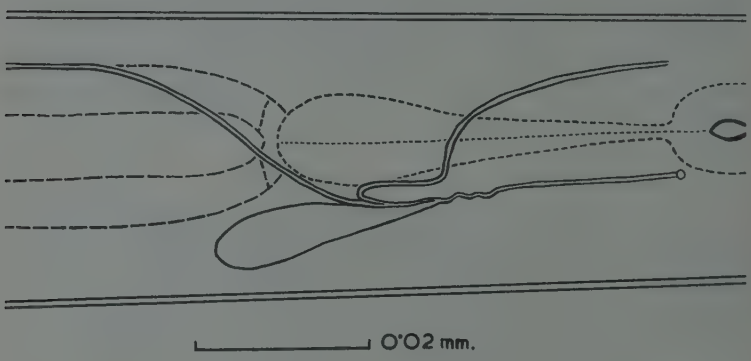

Fig. 1. *Paraphelenchus myceliophthorus*. Ventral view, to show relationships of excretory system.

now seen in a specimen of *Dolichodorus heterocephalus*, from the nerve ring encircling the oesophageal isthmus, nerve fibres passing laterally and posteriorly and running into the hemizonid as it encircles the ventral half of the body between the cuticle and hypodermis. Traces of this connection suggest themselves in some other nematode specimens but only in this one specimen has the system been seen clearly.

This identity throws more light on the structures figured by Hirschmann (1956), in her Fig. 9b, on the head end of *Heterodera glycines* which she likened to the hemizonid. It seems very likely that these are also nerve commissures encircling that part of the body between cuticle and hypodermis.

Thorne (1955), studying *Hemicycliophora*, found that, in general,

the hemizonid was present consistently only in the two bisexual species *H. typica* and *H. penetrans*, though he observed traces of it in two other monosexual species *H. obesa* and *H. tenuis*. Luc (1958) has similar observations for *H. oostenbrinki*, *H. paradoxa* and *H. pauciannulata*. Thorne suggested that the hemizonid might possibly be associated with the sexual process. However, in *Aphelenchus avenae*, which is almost a monosexual species as males are extremely rare, a hemizonid is clearly visible.

The writer is grateful to Dr. Chitwood for expressing his opinions on these two points.

ZUSAMMENFASSUNG

Die weitere Untersuchung des Exkretionssystems bei *Paraphelenchus myceliophthorus* zeigt das Vorhandensein eines vorderen und eines hinteren Röhrchens an dem rechten seitlichen Strang, die sich mit dem Exkretionsgang an der Verbindungstelle mit der ventralen Zelle vereinigen.

Das Hemizonid ist eine ventro-laterale Kommissur des Nervensystems.

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V INTERNATIONAL NEMATOLOGY SYMPOSIUM

As previously announced the Symposium will take place at Uppsala, Sweden. The locality will be Lecture room 9 in the University Building. The preliminary program is as follows:

Sunday, August 9: 7 p.m. Informal pre-symposium meeting.

Monday, August 10: 10 a.m. — 12 noon. Opening speeches, general lectures, information, photographing. *12 noon — 1.30 p.m.* Luncheon. *1.30 p.m. — 4 p.m.* Special lectures. In the evening sightseeing: Old Uppsala and Hammarby, the country home of Linnaeus.

Tuesday, August 11: 9 a.m. — 1 p.m. Lectures (10.45-11.15 tea or coffee). *1 p.m. — 2 p.m.* Luncheon. *2.15 p.m.* Excursion by bus to Ultuna. Visits at the Phytopathological Institute of the Royal Agricultural College (director Prof. K. Björling, demonstrator Dr. F. Ossiannilsson) and at the branch station of the Swedish Seed Association (director Dr. S. Bingefors). Dinner will be taken in the restaurant of the College.

Wednesday, August 12: 9 a.m. — 1 p.m. Lectures (10.45-11.15 tea or coffee). *1 p.m. — 2.30 p.m.* Luncheon. *2.30 p.m. — 5 p.m.* Lectures, discussion of Society matters. Evening free.

Thursday, August 13: 8.30 a.m. Excursion by bus to Stockholm (via Sigtuna). Demonstration in the field. Luncheon. Visit at the Swedish State Plant Protection Institute (director Prof. I. Granhall, div. of zoology Dr. O. Ahlberg, div. of botany Dr. D. Lihnell). The symposium is intended to end with dinner in Stockholm or the neighbourhood.

The participation fee will be 10.— Sw. crs for members of the Society and 20.— Sw. crs for other persons. Accompanying family members free.

Certificate for reduced-fare tickets will be sent if thus is ordered in the application form, which has already been sent out. On presentation of such a certificate at any tourists' agency or travel bureau selling tickets to the Swedish railways the participants and their family members will be granted a 25% reduction of the fare within Sweden to and from Uppsala, the condition being only that at least 50 participants are going by train.

The application form together with the participation fee should be sent to Dr. O. Ahlberg, Statens Växtskyddsanstalt, *Solna 7*, Sweden (New address!) not later than May 15.

Since the earliest possible morning trains from the south will arrive at Uppsala rather late in the day the participants are advised to arrange for arrival on Sunday, August 9.

Nematologica 4 (1959): 161-164. E. J. Brill, Leiden

INCREASING THE RATE OF LARVAL EMERGENCE FROM CYSTS IN HATCHING TESTS WITH BEET EELWORM, *HETERODERA SCHACHTII* SCHMIDT

BY

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The standard method of conducting hatching tests on cyst-forming nematodes (*Heterodera* spp.), devised by FENWICK & WIDDOWSON (1958), is to set up batches of 100 cysts in about 1 ml of hatching medium in solid watch glasses. With this method, the cysts sink after a short period and lie beneath about 1 cm of liquid. This not only decreases the oxygen supply to the cysts, which affects emergence of larvae (WALLACE, 1955), but also makes conditions poor for the larvae to move out of the cysts (WALLACE, *in litt.*).

DEN OUDEN (1954) described a method for bulk-hatching of larvae by placing many cysts on filter paper supported on glass rings, in a Petri dish containing hatching medium. In this way, the cysts are given better aeration and the larvae pass through the filter paper into the liquid and can be recovered for counting.

A method of providing improved aeration which is more convenient for routine hatching tests involves the use of small sieves, made from nylon material and polythene tubing (Fig. 1). By placing batches of cysts on these, the rate of emergence of the larvae can be substantially increased. The sieves are made from two pieces of tubing; one end of the lower section is softened by warming in boiling water and the upper section, with the nylon material held over it, is then pushed into the softened end. Two holes are punctured in the lower section, just below the sieve plate, to aerate the under surface. The sieve is placed in a specimen tube, the cork of which has slits to allow the passage of air, and the sieve plate is kept moist by a wick of sewing cotton, which dips into hatching medium in the bottom of the tube. As the larvae emerge they move down the wick into the liquid and are separated from the cysts, so that competition for oxygen in the immediate neigbourhood of the cysts is reduced. Moreover, the larvae have better conditions for moving out of the cysts. When counts are made, any larvae remaining

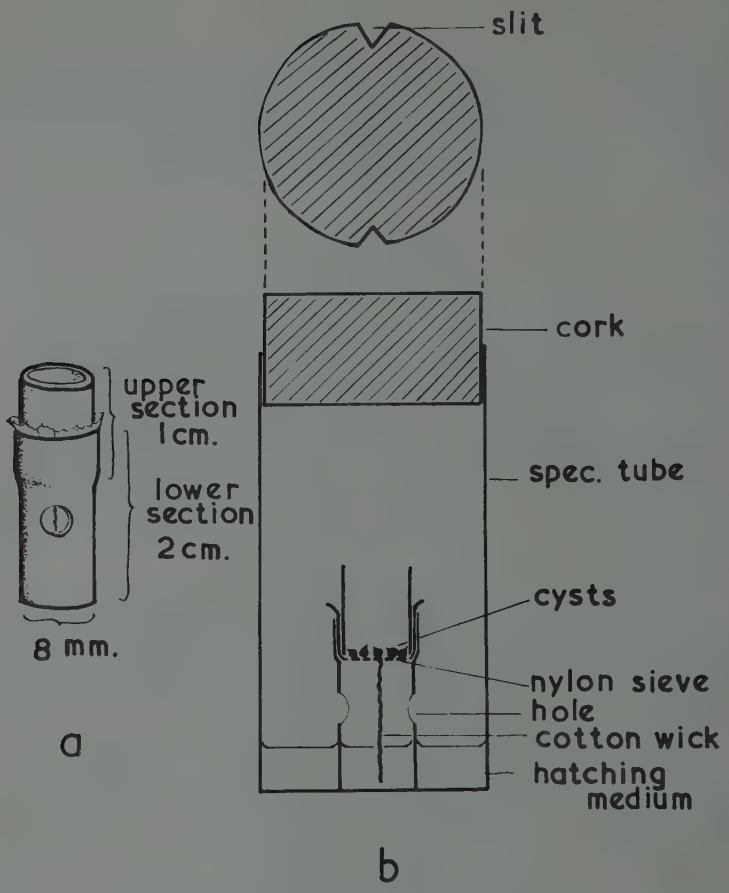

Fig. 1. Polythene and nylon sieve for use in hatching tests with cyst-forming nematodes. a. Sieve in solid view. b. Sectional view of sieve set up for hatching test.

on the sieve are easily washed down into the tube with water from a wash bottle. The larvae are then washed out of the tube for counting and the sieve, with the cysts, replaced in the tube with fresh hatching medium for a further period of hatching.

In this method, then, the cysts are exposed to the air both from above and below, and are surrounded by a thin film of water only, which is much less a barrier to the diffusion of oxygen than the depth of liquid in a watch glass. *Heterodera schachtii* give, after two weeks, a cumula-

tive hatch up to four times greater than by the standard technique; when the test is taken to completion, that is until no more larvae emerge (after 9 to 11 weeks), the total hatch is not significantly different in the two methods (Fig. 2). In the experiments illustrated in the graph,

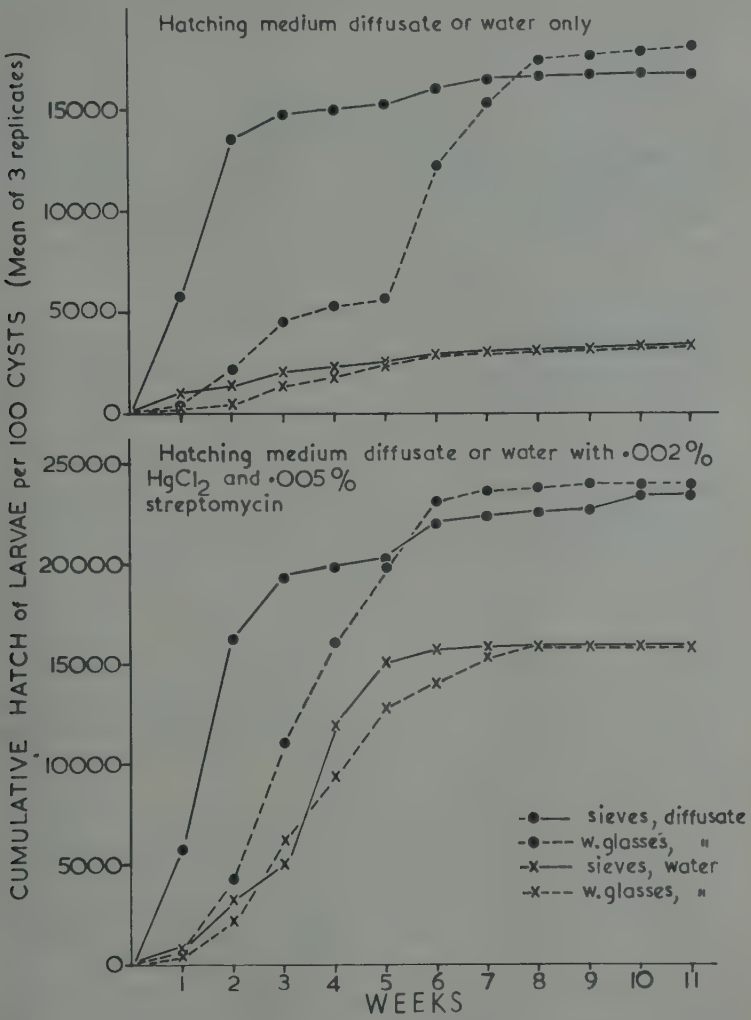

Fig. 2. Comparison of larval emergence from cysts of *H. schachtii* in watch glasses and on sieves, with beet root diffusate and water as hatching media, with and without the addition of mercuric chloride and streptomycin.

from 80% to 90% of the total hatch had occurred by the third week on sieves, whereas this percentage was reached only after five to seven weeks in watch glasses. The emergence rate in water is also slightly increased when sieves are used.

Fig. 2 also shows the effect of adding fungistatic and bacteriostatic substances to the hatching medium. It was thought that decreasing the competition for oxygen by suppressing other micro-organisms might further increase the emergence rate. Addition of 20 p.p.m. mercuric chloride and 50 p.p.m. streptomycin greatly increased both the rate of emergence and the total number of larvae which emerged (for all treatments), although with diffusate the rate using sieves was still proportionally much higher than with watch glasses. It is difficult to determine, however, whether this increase is entirely attributable to the suppression of micro-organisms, including pathogens, or to a direct stimulation of the eggs by the substances added, but probably both these factors played a part.

I should like to thank Miss V. Gibson for technical assistance.

ZUSAMMENFASSUNG

Steigerung des Larvenschlupfes aus den Cysten bei Schlüpfversuchen mit dem Rübennematoden, Heterodera schachtii *Schmidt.*

Im Vergleich zur standardisierten Schlüpftechnik in Gläsern lässt sich eine beträchtliche Steigerung des Larvenschlupfes aus den Cysten von *Heterodera schachtii* durch Verwendung kleiner aus Polyäthylen und Nylon hergestellter Siebe erzielen, in denen die Cysten von allen Seiten aus belüftet werden können. Auf diese Weise kann nach zwei Wochen eine Steigerung um das 4-fache erreicht werden.

Der Larvenschlupf in Wasser wird auch durch Benutzung von Sieben leicht gesteigert. Die gesamte Schlüpfmenge ist jedoch bei beiden Verfahren nicht signifikant unterschiedlich. Die Zugabe fungistatischer und bakteriostatischer Stoffe zum Schlüpfmedium führt zu einem weiteren Ansteigen sowohl der Schlüpfhöhe wie des Gesamtschlupfes. Es ist schwer zu sagen, ob dies einer Unterdrückung O_2-liebender Mikroorganismen zuzuschreiben ist oder einem unmittelbar wirkenden Schlüpfreiz.

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ZUM PROBLEM DER WIRTSFINDUNG PHYTOPATHOGENER NEMATODEN

VON

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In den Untersuchungen von WIESER (1955, 1956) und WALLACE (1958) wurde festgestellt, dass sich die Mehrzahl der Larven von *Meloidogyne hapla* Chitw. bzw. *Heterodera*-Arten nach einer bestimmten Zeit in der mit Pflanzen versehenen Hälfte eines Versuchsgefässes befand. Da über den zeitlichen Verlauf der Anreicherung von Nematoden im Wurzelbereich von Wirtspflanzen noch keine Angaben vorliegen, wurden mit Larven von *Heterodera rostochiensis* Wr. Versuche angestellt, deren Ergebnisse kurz dargestellt und besprochen werden sollen.

Methode: Über die Versuchsanordnung gibt Abb. 1 Aufschluss. Zwei 5 cm lange und 2 cm breite Streifen von Wattepapier (k und q) wurden

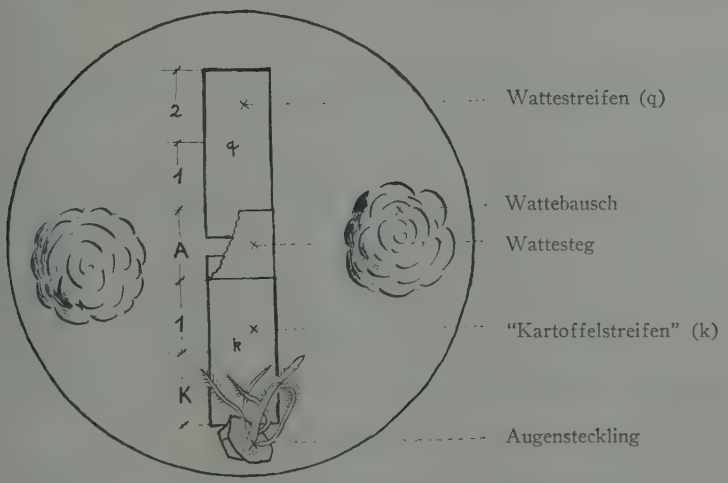

Abb. 1. Schema der Versuchsanordnung — Petrischale mit Wattestreifen und Augensteckling.

hintereinander quer durch eine grössere Petrischale gelegt und feucht gehalten. Die Lücke zwischen den beiden Streifen wurde durch einen Wattesteg (A) überbrückt. Auf den Wattestreifen (k) der einen Seite, unmittelbar am Rande der Schale, kam ein Augensteckling der Kartoffelsorte „Johanna", dessen austreibende Wurzeln die Quelle eines Reizstoffes sein konnten. Der Wattestreifen (q) der anderen Seite blieb unbelegt.

Die Schalen standen in einem Thermostaten bei 21,5° C in vollständiger Dunkelheit. Wassergetränkte Wattebäusche sorgten für hohe, gleichmässige Luftfeuchtigkeit. Es wurden nur solche Schalen mit Nematoden versehen, in denen die Wurzeln nicht über die Hälfte des „Kartoffelstreifens" (k) hinausgewachsen und in denen die Kartoffelstückchen nicht verpilzt oder verfault waren.

Freie, sich schlängelnde Kartoffelnematodenlarven wurden mit wenig Wasser auf den Verbindungssteg (A) aufgetragen. Die Schalen kamen darauf für eine bestimmte Zeit in den Thermostaten zurück. Nach Ablauf der gewünschten Frist wurden die beiden Wattestreifen durch Abheben des Steges (A) voneinander isoliert und die Nematoden im Baermanntrichter von der Watte getrennt und ausgezählt. Die im Wattesteg (A) verbleibenden Tiere und die wenigen Larven, die auf dem Boden der Petrischale haften bleiben, wurden bei der Auszählung nicht berücksichtigt. Als Kontrolle lief ein Versuch ohne Augenstecklinge.

ERGEBNISSE

Die Tabelle I zeigt, dass bei einer Wanderung ohne Einfluss von Kartoffelwurzeln im Mittel 50,3% aller Tiere in einer vorher bezeichneten Hälfte (k') der Schale zu treffen waren. Theoretisch sind 50% zu erwarten. Die stark streuenden Einzelwerte dieser Versuchsreihe zeigen, dass die Streuung in den übrigen Reihen ebenfalls nur dem Zufall unterliegt und nicht auf den Einfluss unterschiedlich wirkender Augenstecklinge zurückzuführen ist. Die Ergebnisse der Wanderversuche mit einseitig aufgelegten Augenstecklingen (Tabelle II und III) zeigen, dass diese einen Einfluss auf die Verteilung in Abhängigkeit von der Zeit ausüben. Die Differenz M_2-M_3 grenzt mit einer Sicherung von 1% ($t = 2{,}75$ bei $(n_2 + n_3) - 2 = 58$) die beiden Werte deutlich voneinander ab (Weber 1948).

DISKUSSION

Die Werte der Tabellen II und III zeigen, dass mit zunehmender Zeit immer mehr Nematoden in der Nähe der Pflanzen gefunden wer-

TABELLE I

Verteilung ohne Einfluss von Kartoffelwurzeln nach 24 Stunden

lfd Nr.	n	k' in % von n	± m
1	63	1,6	1,60
2	151	2,0	1,01
3	84	4,7	2,33
4	105	8,6	2,74
5	90	10,0	3,16
6	116	10,3	2,82
7	82	25,0	4,90
8	174	26,6	3,30
9	129	27,9	3,94
10	86	27,9	4.84
11	120	34,2	4,34
12	87	37,9	5,21
13	102	38,2	4,82
14	96	53,1	5,10
15	268	58,5	3,02
16	119	59,6	4,50
17	121	62,4	4,40
18	123	65,8	4,33
19	96	66,4	4,96
20	76	68,4	5,28
21	114	68,9	4,30
22	180	73,3	3,30
23	102	76,5	4,20
24	123	80,5	3,57
25	120	82,5	3,47
26	69	82,6	4,56
27	111	82,8	3,58
28	116	85,4	3,28
29	112	87,6	3,12
30	87	95,2	2,33

$M_1 = 50{,}3\%$ $\sigma_1 = 30{,}05$ $m_1 = 5{,}5$

TABELLE II

Verteilung unter Einfluss von Kartoffelwurzeln nach 6 Stunden

lfd Nr.	n	k in % von n	± m
1	221	15,4	2,43
2	221	16,3	2,48
3	140	27,1	3,75
4	133	29,3	3,95
5	587	38,3	2,00
6	1482	41,9	1,28
7	2413	42,0	1,00
8	619	45,4	2,00
9	134	47,8	4,19
10	232	50,4	3,28
11	3096	52,5	0,90
12	2139	54,9	1,08
13	147	55,4	4,10
14	121	55,4	4,52
15	227	59,9	3,25
16	109	60,6	4,68
17	126	61,9	4,33
18	153	65,4	3,84
19	778	66,1	1,70
20	182	66,5	3,50
21	186	66,7	3,46
22	782	70,0	1,64
23	208	70,2	3,17
24	181	74,0	3,26
25	197	76,6	3,16
26	2876	76,8	0.79
27	114	77,2	3,92
28	213	81,7	2,65
29	165	81,8	3,00
30	162	97,7	1,18

$M_2 = 58{,}0\%$ $\sigma_2 = 19{,}5$ $m_2 = 3{,}56$

TABELLE III

Verteilung unter Einfluss von Kartoffelwurzeln nach 24 Stunden

lfd Nr.	n	k in % von n	± m
1	923	32,5	1,54
2	377	33,2	2,43
3	2102	46,9	1,09
4	572	50,9	2,09
5	221	52,5	3,36
6	284	59,9	2,91
7	946	60,1	1,59
8	4177	60,9	0,75
9	1188	62,4	1,47
10	196	63,3	3,44
11	1812	66,9	1,11
12	441	68,9	2,20
13	4380	70,2	0,69
14	754	71,9	1,64
15	1601	71,9	1,12
16	532	72,9	1,92
17	827	73,9	1,53
18	2014	76,0	0,96
19	3747	76,3	0,70
20	517	77,4	1,83
21	1461	78,4	1,08
22	128	79,7	3,56
23	1275	82,0	1,08
24	685	82,5	1,44
25	1246	83,9	1,04
26	721	87,2	1,24
27	112	89,3	2,92
28	2519	92,4	0,53
29	325	95,1	1,20
30	8940	96,7	0,19

$M_3 = 70{,}6\%$ $\sigma_3 = 16{,}3$ $m_3 = 2{,}97$

n = Anzahl der Tiere im Versuch k = Wattestreifen mit Augensteckling k' = Wattestreifen ohne Augensteckling (im Kontrollversuch) m_1; m_2; m_3 = mittlere Abweichungen der Mittelwerte M_1; M_2; M_3 (Berechnung der Werte von M; σ und m nach dem Summenverfahren).

den. Die bisher allgemein als gültig anerkannte Lockstofftheorie nimmt an, dass die Nematoden chemotaktisch von ihren Wirtspflanzen angelockt werden, dem Konzentrationsgefälle spezifischer Stoffe entgegenwandern und so die ihnen günstigen Pflanzen erreichen (BAUNACKE, 1922 u.a.). Die in den angeführten Versuchen nachgewiesene Zeitabhängigkeit der Ansammlung im Wurzelbereich lässt jedoch noch eine andere Deutung der Wirtsfindung zu, die zugleich der starken Streuung der Einzelwerte gerecht wird.

Wenn man annimmt, dass die Larven unter den Bedingungen des oben geschilderten Versuches mit einer mittleren Wandergeschwindig-

TABELLE IV

Verteilung ungerichtet wandernder Larven bei Festlegung im Köder (K)

Zeit in Stunden	k			q		% in k (k + q = 100)	
	K	1	A	1	2		
0	0	0	160	0	0	0	
1	0	40	80	40	0	50,0	
2	10	40	60	40	10	50,0	
3	20	35	50	37	18	50,0	
4	29	30	43	36	22	50,4	
5	36	26	38	34	26	50,8	
6	43	22	34	33	28	51,6	M_2 theor.
7	49	20	30	32	29	53,1	
8	54	17	28	30	29	54,6	
9	58	15	26	29	29	55,7	
10	62	14	24	28	29	57,1	
11	66	13	23	27	29	58,5	
12	69	12	21	27	29	59,1	
13	72	12	21	26	28	60,9	
14	75	11	19	25	28	61,9	
15	78	10	19	24	27	63,3	
16	80	10	18	24	26	64,3	
17	82	10	18	23	26	65,2	
18	85	10	17	22	26	66,4	
19	87	9	17	22	25	67,1	
20	89	9	16	21	24	68,5	
21	91	9	16	20	23	69,9	
22	93	8	15	20	22	70,6	
23	95	8	15	19	22	71,5	
24	97	8	14	19	21	72,4	M_3 theor.

k = Wattestreifen mit Augensteckling

q = Wattestreifen ohne Augensteckling

A = Auftragungsort der Larven

K = Von Kartoffelwurzeln durchwachsener Teil des Wattestreifen k

M theor. = theoretisch zu erwartender Anteil der Larven im Wattestreifen mit Augensteckling nach einer bestimmten Zeit.

keit von 2 cm/h [1]) unbeeinflusst von einem richtenden Reiz der Kartoffelwurzel ihren Weg nehmen, so werden Tiere zufällig die Wurzeln (K) berühren und versuchen, in diese einzudringen. Ein Teil der Tiere wird also aus dem Zufallspiel der Bewegungen herausgenommen und stellt in Wurzelnähe seine Wanderung ein. Unter diesen Voraussetzungen lässt sich eine zu erwartende Unregelmässigkeit der Verteilung berechnen.

Der Tabelle IV liegt folgende Berechung der Verteilung zugrunde: In der Zeiteinheit von 1 Stunde legt jedes der Tiere 2 cm in irgendeiner Richtung zurück. In jedem der Quadrate 2, 1, A und 1 (vergl. Abb. 1) wird — grosse Zahlen vorausgesetzt — ein Viertel der Tiere an jedem Rande umkehren und je ein Viertel in das Nachbarquadrat gelangen. Im Quadrat K werden alle Tiere festgehalten. Es wandern wohl Tiere ein, aber keines verlässt den Wurzelbereich. Die Tabelle zeigt, dass die Zahl der Tiere im Wurzelbereich (K) im Laufe der Zeit zunehmen muss, ohne dass die Larven diesen Bereich bevorzugt aufsuchen. Während nach 6 Stunden erst 51,6% (= M_2 theor.) aller Tiere im „Kartoffelstreifen" (k = 1 + K) zu erwarten sind, sind es nach 24 Stunden bereits 72,4% (= M_3 theor.). Es wird eine Anlockung nur vorgetäuscht.

Der t-Wert der Differenz M_2-M_2 theor. beträgt 1,6 während der der Differenz M_3-M_3 theor. nur 0,6 beträgt. In beiden Fällen liegt der theoretische Mittelwert im gesicherten Bereich des experimentellen; beide sind — zumindest mit grosser Wahrscheinlichkeit — identisch.

Die Übereinstimmung der theoretischen Erwartung und der empirischen Befunde bestätigen die Annahme einer ungerichteten Wanderung der Kartoffelnematodenlarven und ein Verbleiben derselben im zufällig erreichten Wurzelbereich.

Unter den im Boden gegebenen Diffusionsverhältnissen — geringe Diffusionsgeschwindigkeit auf Grund des Verdünnungsgrades, Fehlen eines homogenen Diffusionsmediums, Bewegungen des Bodenwassers, Abbau von Stoffen durch Mikroorganismen, Adsorption an Bodenkolloide, Überschneidung von Teilgefällen, usw. — erscheint das Vorkommen von diffus wirkenden Reizen wahrscheinlicher als das Vorhanden-

1) Die Wandergeschwindigkeit betrug im Mittel von 200 Einzelmessungen bei Zimmertemperatur auf sandbestreuten Agarplatten bei direkter Messung längs eines Weges von 0,75 mm 6 cm/h; in kompakt gelagertem feuchtem Sand und in feuchten Wattestreifen bei indirekter Beobachtung maximal 4 bis 5 cm/h. Da der effektiv in einer Richtung zurückgelegte Weg bei zufälliger Verteilung der Quadratwurzel des tatsächlich zurückgelegten Weges entspricht, kann man mit 2 cm/h mittlerer Wandergeschwindigkeit unter den Versuchsbedingungen rechnen.

sein — bisher noch unbewiesener — weitreichender, harmonisch aufgebauter Diffusionsgefälle.

Es wird daher der mit graduierten Gefällen rechnenden Lockstofftheorie folgende Hypothese gegenübergestellt:

Die freien Larven des Kartoffelnematoden treffen zufällig auf eine Kartoffelwurzel. Hier dringen sie ein oder werden durch einen ausserhalb der Wurzel wirkenden, diffusen Reiz in ihrer Beweglichkeit beeinträchtigt, so dass sie den Wurzelbereich nur langsam oder gar nicht verlassen können. Die Pflanze und ihre Ausscheidungen haben keinen richtenden Einfluss auf die freien Larven, sondern beeinflussen lediglich deren Wandergeschwindigkeit.

Wenn man in „Beeinflussung der Wandergeschwindigkeit" auch eine Erhöhung der Beweglichkeit im Wurzelbereich einschliesst, dann erklärt vorstehende Hypothese auch die von Wieser (1956a) beobachtete Abstossung. Es werden, wenn die Bewegungen der Tiere im Wurzelbereich unter dem Einfluss eines Reizes beschleunigt werden, in der gleichen Zeit mehr Tiere den Wurzelbereich verlassen als ihn zufällig berühren. Nach Berechnungen, die ähnlich wie in Tabelle IV ausgeführt wurden, sind in 24 Stunden nur noch etwa 35% bis 40% aller Tiere in Pflanzennähe zu erwarten.

Die Beeinflussung der Bewegungsgeschwindigkeit durch verschiedene Intensitäten eines diffusen Reizes wird von Fraenkel & Gunn (1940) als Orthokinese bezeichnet, so dass man vorstehende Hypothese als die der chemo-orthokinetischen Wirtsfindung bezeichnen kann.

SUMMARY

The fact that the number of larvae of *H. rostochiensis* on the root side of a Petri-dish increase with time (58% after 6 hours, 70,6% after 24 hours) agrees with the calculation that nematodes wander at random. Reasons are given which suggest that the building up of diffusion gradients of an attractant substance in soil is almost impossible. The following hypothesis of chemo-orthokinetical host-finding is put forward as a possible explanation of congregation near host roots: nematode larvae meet roots by chance and enter them or alter their movement under influence of substances emitted by roots. Larvae do not leave the rhizosphere and so accumulate there. Plants and their diffusates do not directly attract nematodes chemotropically but solely influence the rate of movement of larvae.

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CORRECTION

TH. SALENTINY: Durch die Rübenrasse des Stockälchens *Ditylenchus dipsaci* hervorgerufene Schadbilder bei einigen Unkrautarten. *Nematologica* **4** (2): 142-146, muss es bei Abb. 3: Befall bei *Thlaspi arvense* (anstatt *Anagallis arvensis*) heissen.

Nematologica 4 (1959): 172-186. E. J. Brill, Leiden

EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE WANDERUNG VON NEMATODEN

VON

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Die Frage, wie weit pflanzenparasitäre Nematoden wandern, um eine geeignete Wirtspflanze zu finden, ist trotz etlicher Untersuchungen noch nicht hinreichend geklärt. Selbst bei dem Verhalten einiger *Heterodera*-Arten, das noch am besten untersucht ist, schwanken die Ergebnisse sehr. So wechseln z.B. die Angaben über die bei *H. schachtii* in einer Generation zurückgelegten Entfernungen zwischen 0,56 m (MÜLLER & MOLZ 1914) und 18 m (KÜHN 1883). Über andere Nematoden liegen nur wenige Angaben vor, die sich meist nicht auf Versuche, sondern auf Feldbeobachtungen gründen.

In Weiterführung einiger Versuche von GOFFART (1950) sollte das Wanderungsverhalten einiger Nematoden in verschiedenen Versuchsanordnungen geprüft werden. Dabei wurden die komplexen Verhältnisse in natürlichem Boden schrittweise bis auf einfache, der Beobachtung leichter zugängliche Bedingungen reduziert. Wegen der besseren Vergleichbarkeit mit den Angaben anderer Autoren wurden die meisten Versuche mit den verhältnismässig gut bekannten Kartoffel- und Rübennematoden durchgeführt. Die ausserdem mit *Ditylenchus dipsaci* und *Paratylenchus sp.* gemachten Untersuchungen dienen in erster Linie zur Ergänzung. Sie sollen später weitergeführt werden.

VERSUCHE MIT NATÜRLICHEM BODEN UND WIRTSPFLANZEN

Die ersten Versuche erfolgten in Anlehnung an BAUNACKE (1922) in Holzkästen (20 cm breit, 14 cm hoch, 60 cm lang). Die mit sterilisierter Erde gefüllten Kästen wurden an der Stirnseite mit je 100 Zysten von *Heterodera rostochiensis* oder *H. schachtii* infiziert und dann mit Wirtspflanzen (Kartoffeln oder Zuckerrüben) besetzt. Dabei ergaben sich verschiedene Gruppen mit unterschiedlicher Entfernung zwischen Infektionsstelle und Wirtspflanze. Die Entfernung betrug 10,

30 oder 50 cm; in einer Serie wurden die Kästen in allen drei Abständen mit Pflanzen besetzt. Als Kontrollen dienten einmal Kästen ohne Zysten, zum anderen Kästen, bei denen die Zysten unmittelbar an den Pflanzen in die Erde kamen. Die Kästen wurden während des Versuches (5 Monate) sehr vorsichtig gegossen, damit keine Zysten oder Larven weggespült werden konnten. Am Ende der Vegetationsperiode (Oktober) wurde die vorher in Scheiben von 2 cm Dicke zerlegte Erde der Kästen mit der Fenwickkanne auf Zysten untersucht. Die dabei gefundenen Zysten waren ein Mass für die von den Larven zurückgelegten Entfernungen, wobei die durch das Wurzelwachstum nach dem Eindringen der Tiere verursachten Verschiebungen nicht erfasst werden konnten.

Tabelle I

Anzahl der in bestimmter Entfernung von der Infektionsstelle gefundenen neuen Zysten von Heterodera rostochiensis

Abstand der Probe von Infektionsstelle	Abstand der Pflanzen von Infektionsstelle				Kontrolle	
	10	30	50	10, 30, 50 cm	10, 30, 50 cm direkt inf.	ohne Zysten
4- 6 cm	320	17	0	80	—	0
8-10	56	3	0	6	1200	0
12-14	7	7	1	5	1000	0
16-18	2	0	4	10	150	0
20-22	0	0	4	5	710	0
24-26	0	0	0	1	2600	0
28-30	0	0	0	0	1700	0
32-34	0	0	0	0	400	0
36-38	0	0	0	0	30	0
40-42	0	0	0	0	700	0
44-46	0	0	0	0	2400	0
48-50	0	0	0	0	1900	0
52-54	0	0	0	0	300	0
56-58	0	0	0	0	—	0

In Tabelle I ist ein Teil der Ergebnisse zusammengestellt. Spalte 1 dieser Tabelle enthält die Angaben über die Entfernungen zwischen Infektionsstelle und Ort der Probenahme, in den übrigen Spalten steht die Anzahl der in den einzelnen Versuchsreihen in diesen Proben gefundenen Zysten. Die Spalten 6 und 7 beziehen sich auf die Kontrollen, wobei 7 die unverseuchte und 6 die an jeder Pflanze direkt infizierte Versuchsreihe wiedergibt. Die Tabelle zeigt, dass in den eigentlichen

Versuchsreihen (Spalten 2-5) nur ganz wenige Zysten gefunden wurden.

Eine Nachprüfung der Infektionszysten ergab, dass nur dann, wenn die Wirtspflanzen nahe an der Infektionsstelle standen, ein stärkeres Schlüpfen der Larven stattgefunden hatte. Schon bei einer Entfernung von 30 cm waren die Zysten an der Infektionsstelle praktisch noch voll. Ein Schlüpfversuch in vitro zeigte, dass die Larven voll lebensfähig waren. Im Boden wird das Schlüpfen offenbar nur dann in stärkerem Masse ausgelöst, wenn die Wurzeln sehr nahe an die Zysten herankommen. Eine Aktivierung und Anlockung, wie sie u.a. BAUNACKE (1922) annahm, konnte nicht festgestellt werden. Die meisten Larven legten Strecken unter 20 cm Länge zurück. Grössere Entfernungen wurden nur ganz vereinzelt überwunden.

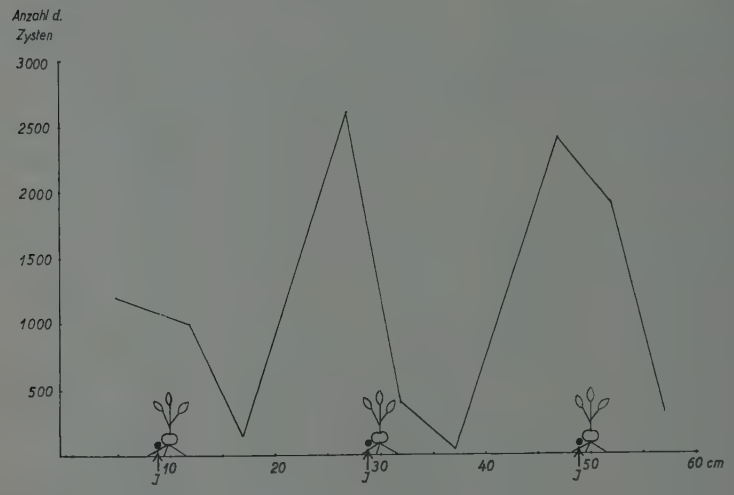

Abb. 1. Verteilung der neugebildeten Zysten von *Heterodera rostochiensis* im Boden bei Infektion in unmittelbarer Nähe der Pflanzen. J = Infiziert mit 100 Zysten.

Die Larven suchen offenbar nicht lange, sondern dringen in die erste geeignete Wurzel ein, auf die sie stossen. Das geht deutlich aus den Kontrollen hervor, bei denen die Kästen an drei Stellen mit Pflanzen besetzt und direkt an den Pflanzen mit Zysten infiziert waren. Hier haben die Larven, wie Abb. 1 für *H. rostochiensis* zeigt, nur ganz kurze Strecken zurückgelegt. Schon wenige cm von der Infektionsstelle sinkt die Zahl der neugebildeten Zysten stark ab, obwohl natürlich auch

hier sehr viele Wurzeln waren. Ähnliche Beobachtungen wurden auch in anderem Zusammenhang bei verschiedenen Versuchen gemacht. Die Larven von *H. schachtii* verhielten sich grundsätzlich ähnlich.

Nachdem unter den vorerwähnten Bedingungen kein starkes Wandern beobachtet werden konnte, wurde der ganze Versuch wiederholt mit der Änderung, dass die Infektionsstelle drei Wochen lang alle zwei Tage mit einigen ccm Wurzelablaufwasser gut wachsender Kartoffel- bzw. Rübenpflanzen begossen wurde. Damit sollte versucht werden, die Larven zu einem stärkeren Schlüpfen und eventuell auch zu einem verstärkten Wandern zu veranlassen. Die Ergebnisse der Auswertung nach der Vegetationsperiode, die für den Kartoffelnematoden in der Tabelle II zusammengestellt sind, zeigten, dass jetzt Zysten wirklich allgemein zahlreicher und auch in grösserer Entfernung auftraten als im ersten Versuch. Die Zugabe des Ablaufwassers hatte also die Larven zu einer stärkeren Fortbewegung veranlasst. Dabei war aber noch nicht zu entscheiden, ob dieses auf eine ungerichtete Steigerung der Aktivität zurückzuführen war oder auf eine Sensibilisierung gegenüber der vermuteten anlockenden Wirkung der Wurzeln. Daher wurde eine neue Versuchsreihe mit einer anderen Anordnung angesetzt.

Tabelle II

Anzahl der in bestimmter Entfernung von der Infektionsstelle gefundenen neuen Zysten von Heterodera rostochiensis *bei Begiessen der Infektionsstelle mit Ablaufwasser*

Abstand der Probe von Infektionsstelle	Abstand der Pflanzen von Infektionsstelle					
					Kontrolle	
	10	30	50	10, 30, 50 cm	10, 30, 50 cm direkt inf.	ohne Zysten
4- 6 cm	175	90	0	124	—	0
8-10	22	61	0	62	1000	0
12-14	8	19	4	6	1350	0
16-18	3	5	30	0	220	0
20-22	0	4	24	0	180	0
24-26	0	2	8	6	370	0
28-30	0	0	4	4	490	0
32-34	0	0	0	11	1650	0
36-38	0	0	0	0	550	0
40-42	0	0	4	0	500	0
44-46	0	2	14	0	1000	0
48-50	0	5	1	0	640	0
52-54	0	0	0	0	510	0
56-58	0	0	0	0	—	0

VERSUCHE MIT NATÜRLICHEM BODEN OHNE WIRTSPFLANZEN

In die Mitte kleinerer Holzkästen von 30 cm Länge, 18 cm Breite und 8 cm Höhe, gefüllt mit dampfsterilisierter Erde, kamen jeweils 200 Zysten von Kartoffel- bzw. Rübennematoden. Dann wurden drei Wochen lang alle drei Tage einige ccm Wurzelablaufwasser (in den Kon-

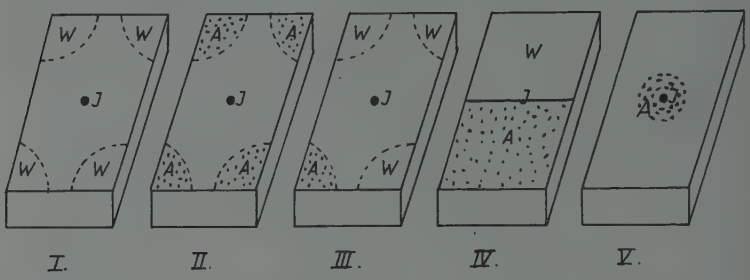

Abb. 2. Schlematische Darstellung der Versuche zur Prüfung der Wirkung von Wurzelablaufwasser auf Larven der Rüben- und Kartoffelnematoden. J = Infiziert mit 200 Zysten, W = Auftropfstelle für Wasser, A. = Auftropfstelle für Ablaufwasser.

trollen Leitungswasser) an bestimmten Stellen aufgetropft (Abb. 2). Wenn wirklich eine über mehr als 10 cm reichende aktivierende und anlockende Wirkung der Wurzelsekrete vorhanden war, konnte sie sich in den Versuchen nach Schema II und V in einer allgemein verstärkten und bei Schema III in einer gerichteten Wanderung der Larven bemerkbar machen. In den Kästen nach Schema IV, in denen das Ablaufwasser bis auf 1-2 cm an die Infektionsstelle gebracht wurde, war eine Bevorzugung der einen, mit Ablaufwasser behandelten Seite zu erwarten.

Drei Wochen nach Versuchsbeginn wurden in festgelegten Abständen von den Infektionstellen in Richtung auf die Auftropfstellen Bodenproben von 3 cm Durchmesser ausgestochen und nach der Methode von SEINHORST (1956) auf Larven untersucht. Wider Erwarten fanden sich in allen Versuchen nur so wenige Tiere, dass die Ergebnisse statistisch nicht zu sichern waren.

In einem anschliessend angesetzten Schlüpfversuch konnte festgestellt werden, dass die Larven in den Infektionszysten voll lebensfähig waren. Daher muss der negative Ausgang der Versuche auf das Fehlen einer ausreichend starken Fernwirkung zurückgeführt werden, selbst wenn man den Abbau der Sekrete im Boden berücksichtigt (FENWICK

1956). Auch die Ergebnisse von Versuch V zeigten, dass zum Schlüpfen der Larven die direkte Berührung mit Wurzelsekreten erforderlich ist. Um zu prüfen, ob wenigstens auf frei im Boden befindliche Larven eine anlockende Wirkung über eine gewisse Entfernung hin feststellbar wäre, wurde der gesamte Versuch mit geschlüpften Larven wiederholt. In jeden Kasten kamen rund 10000 Larven, die zur Beseitigung etwa anhaftender Wurzelsekretreste zweimal in Wasser gewaschen waren. Die Entnahme und Auswertung der Bodenproben erfolgte zwei, vier und sechs Wochen nach Versuchsbeginn. Dabei zeigte sich, dass die Larven tatsächlich in grösserer Anzahl gewandert waren als im ersten Versuch. Eine besondere Richtung wurde im allgemeinen nicht bevorzugt. Die Tiere hatten sich von den Infektionsstellen aus in alle Richtungen bewegt. Nur in den Versuchen nach Schema IV, wo die Larven also in unmittelbarer Nähe des Ablaufwassers waren, machte sich eine gewisse aktivierende und anlockende Wirkung bemerkbar.

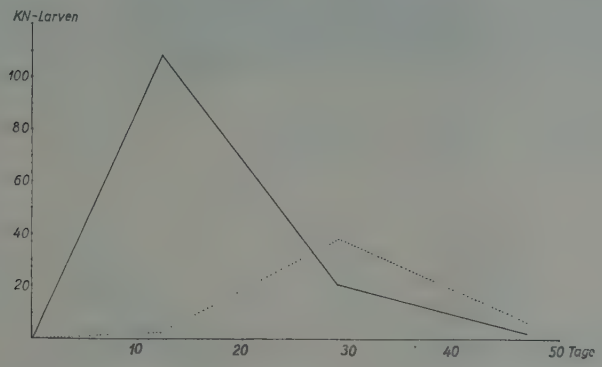

Abb. 3. Anzahl der nach 12, 28 und 47 Tagen in 4 cm Entfernung von der Infektionsstelle gefundenen Larven des Kartoffelnematoden in einem Versuch nach Schema IV der Abb. 2.
— = Boden mit Ablaufwasser behandelt; ... = Boden mit Leitungswasser behandelt.

Die Abb. 3 gibt dafür ein Beispiel. Man sieht, dass die Hauptwanderungswelle der Larven eine bestimmte Entfernung (4 cm) unter dem Einfluss der Wurzelsekrete zahlreicher und etliche Tage eher erreichte als in dem nur mit Wasser behandelten Teil. Die Larven waren also schneller gewandert. Eine anlockende Wirkung könnte man darin sehen, dass in den Bodenproben auf der Ablaufwasserseite im Durchschnitt 46% mehr Larven gefunden wurden als auf der Wasserseite. Diese

Wirkung kann aber nur schwach gewesen sein, denn insgesamt hatten sich nur etwa 15% der in den Proben gefundenen Nematoden (rund 1000 Tiere je Kasten) mehr als 2 cm von der Infektionstelle entfernt.

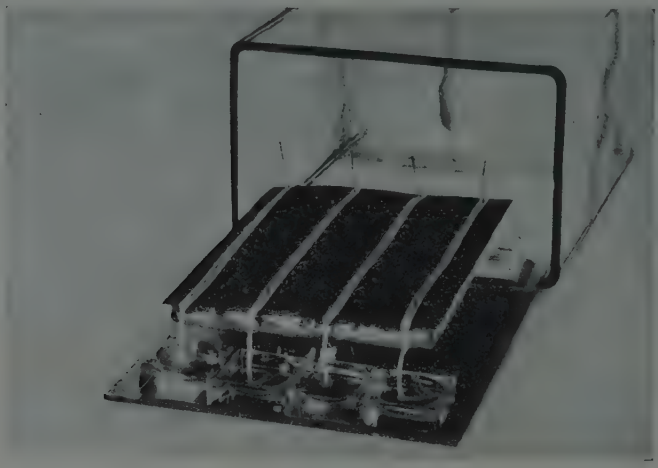

Fig. 4. Versuchsordnung zur Untersuchung der Nematodenwanderung in Filterpapier und in Sand.

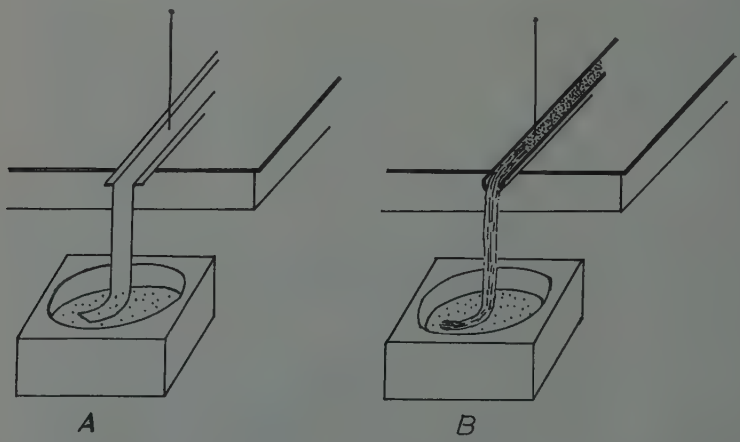

Abb. 5. Schematisch dargestellte Ausschnitte aus der in Abb. 4 wiedergegebenen Versuchsanordnung. A = Filterpapierstreifen, der mit einer Nadel auf einen Kunststoffstreifen geheftet ist und in ein Schälchen mit Wasser bzw. Ablaufwasser reicht. B = Halbierter und mit Sand gefüllter Plastikschlauch, der durch einen dicken Baumwollfaden mit dem flüssigkeitsgefüllten Schälchen verbunden ist.

VERSUCHE MIT KÜNSTLICHEN MEDIEN

Die bisherigen Versuche wurden unter annähernd natürlichen Bedingungen in normalem Boden durchgeführt. Dadurch wirkte eine Reihe chemischer, physikalischer und biologischer Faktoren mit, deren Einfluss auf die Larven im Einzelnen nicht genau zu kontrollieren war. Um das Wanderungsverhalten der Tiere unter möglichst einfachen Bedingungen zu prüfen, wurde eine neuartige Versuchsanordnung entwickelt, in der die störenden Faktoren weitgehend ausgeschaltet waren. Der wichtigste Unterschied ist der, dass die Nematoden nicht in natürlichem Boden wandern, sondern in reinem Sand von bestimmter Körnchengrösse (0,2-0,5 mm) oder in Filterpapier.

Die Anordnung ist in den Abb. 4 und 5 dargestellt. Die wesentlichen Bestandteile sind halbierte, mit Sand gefüllte Plastikschläuche bzw. Filterpapierstreifen, beide 20-25 cm lang. Sie stehen in Verbindung mit Glasschälchen, die mit Wasser oder Ablaufwasser gefüllt werden. Die Schläuche und die Streifen saugen sich dann entsprechend ihrer Kapazität je nach Versuchsanordnung von einer oder von beiden Seiten mit

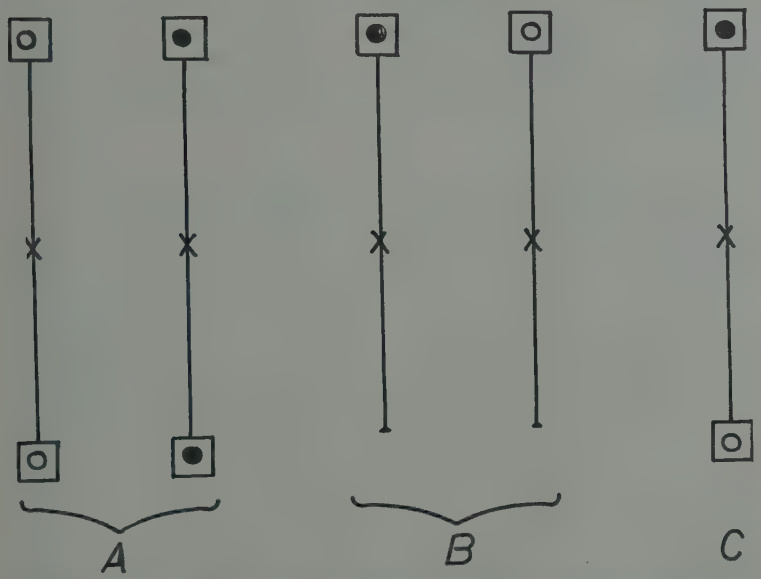

Abb. 6. Schematische Darstellung der verschiedenen Versuchsreihen zur Untersuchung des Wanderungsverhaltens der Nematoden. ⊡ = Schälchen mit Leitungswasser; ⊙ = Schälchen mit Ablaufwasser; X = Infektionsstelle.

Flüssigkeit voll. Die Nematoden werden vorsichtig mit einer Pipette in einem möglichst kleinen Tropfen Wasser auf die Mitte der Bahn getropft, von wo sie dann nach zwei Seiten hin wandern können. Zur Einhaltung konstanter Bedingungen lässt sich die ganze Einrichtung für die Dauer des Versuches in einen abgeschlossenen Glasbehälter bringen. Nach Ablauf der jeweils vorgesehenen Zeit werden die Schläuche oder Streifen in 1 cm lange Stücke geschnitten. Die Anzahl der in jedem Abschnitt vorhandenen Älchen kann dann mikroskopisch leicht bestimmt werden.

In Abb. 6 sind die durchgeführten Versuchsreihen im Schema wiedergegeben. Im allgemeinen haben sich die sandgefüllten Plastikschläuche besser bewährt als die Papierstreifen. Sie erbrachten meist klarere Ergebnisse, was wohl auf die natürlicheren Verhältnisse zurückzuführen ist. Die Hauptnachteile der Filterpapiermethode liegen darin, dass die Nematoden nicht durch das Papier wandern, sondern sich in dem dünnen Wasserfilm fortbewegen, der sich zwischen Papier und Unterlage bildet. Wenn dann das Papier an einer Stelle nicht ganz fest aufliegt, etwa infolge einer kleinen Unebenheit der Unterlage oder einer kleinen Luftblase, treten sofort Stockungen und Hindernisse auf, die das Ergebnis nachteilig beeinflussen. Für die meisten derartigen Versuche ist also Sand vorzuziehen, auch wegen der natürlicheren Feuchtigkeitsverhältnisse. Die im Folgenden aufgeführten Werte beziehen sich, wenn nicht anders angegeben, auf Versuche mit sandgefüllten Plastikschläuchen.

Vor Beginn der Versuche war zunächst zu prüfen, ob die Larven unter diesen Bedingungen überhaupt wandern, und wieweit sie durch das Auftropfen sowie durch eventuell auftretende Strömungen passiv transportiert werden. Daher wurden sämtliche Versuche erst einmal mit lebenden und toten Larven (durch Hitze getötet) durchgetestet. Es konnte einwandfrei festgestellt werden, dass sich die lebenden Nematoden sowohl in den Filterpapierstreifen als auch in dem Sand aktiv von der Infektionsstelle fortbewegten. Bei richtiger Handhabung blieben die Tiere beim Auftropfen sämtlich innerhalb eines Abschnittes von höchstens 1 cm Länge liegen. Alle ausserhalb dieser Stelle gefundenen Älchen müssen also dorthin gewandert sein. Trotzdem wurde bei der Beurteilung des Wanderungsverhaltens in den Versuchen für die Infektionsstelle ein Abschnitt von 2 cm berechnet, um ganz sicher zu gehen.

Über die erforderliche Dauer der einzelnen Versuche bestanden zunächst noch keine bestimmten Vorstellungen, da die in der Literatur angegebenen Wanderungsgeschwindigkeiten von *Heterodera*-Larven

sehr unterschiedlich sind. Die Versuche wurden daher mit Laufzeiten von 1, 3, 5, 7, 14, 21 und 28 Tagen wiederholt.

Dabei stellte sich heraus, dass die Wanderung unter diesen Verhältnissen erst nach etwa 5 Tagen exakt messbar war. Zwar legten einzelne Larven auch schon in kürzerer Zeit Strecken von mehreren Zentimetern zurück, aber die Masse der aufgetropften Tiere entfernte sich nur sehr

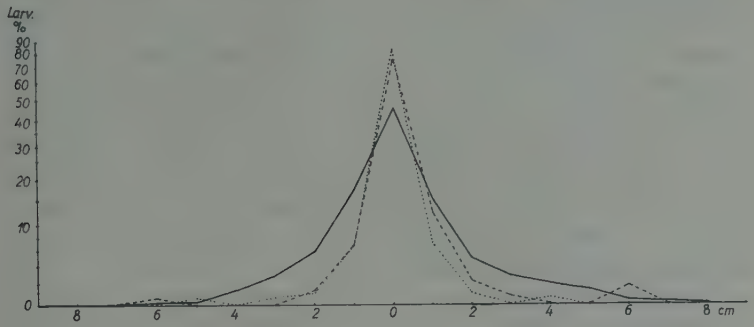

Abb. 7. Die Ausbreitung von Larven des Kartoffelnematoden in feuchtem Sand nach 1 (.....), 3 (- - - -) und 5 (——) Tagen.

langsam von der Auftropfstelle, wie als Beispiel die Abb. 7 zeigt. In fast allen Versuchen mit einer kürzeren Laufzeit als vier Wochen war der grösste Teil der Larven innerhalb des 2 cm langen Abschnittes der Infektionsstelle geblieben. Die sich aus diesen Versuchen ergebende Ausbreitungsgeschwindigkeit ist natürlich nicht gleichzusetzen mit der Wanderungsgeschwindigkeit der einzelnen Larven. Diese ist grösser, da die Tiere in den Versuchen nicht zielgerecht in eine Richtung gehen, sondern oft hin- und herkriechen.

Um eine Vorstellung von der Ausbreitungsgeschwindigkeit anderer pflanzenparasitärer Nematoden zu bekommen, wurden einige Versuche mit *Ditylenchus dipsaci* und *Paratylenchus* spec. durchgeführt. Es ergaben sich für die einzelnen Nematodenarten sehr unterschiedliche Werte. Eine Strecke von 10 cm wurde von Stockälchen in etwa 3 Stunden, von den Larven des Kartoffelnematoden in etwa 3 Tagen und von den Paratylenchen in mehr als 10 Tagen zurückgelegt.

Diese Zahlen sind Durchschnittswerte für grössere Anzahlen von Nematoden. Bei der Beobachtung einzelner Tiere kann man zu anderen Werten kommen, da die individuellen Unterschiede sehr gross sind. Einzelne *Heterodera*-Larven erreichten eine bestimmte Entfernung fast ebenso schnell wie Stockälchen, während umgekehrt etliche Stockälchen

auch nach Tagen erst wenige cm zurückgelegt hatten. WALLACE (1958d) hat gezeigt, dass bei der Fortbewegung in Wasser auch noch die Körperlänge und die Beweglichkeit der Nematoden für die Geschwindigkeit wichtig sind.

In natürlichen Böden dürfte die Ausbreitung aller Nematoden wesentlich langsamer vor sich gehen als unter den vereinfachten Versuchsbedingungen in einem annähernd homogenen Substrat. Die vor allem in der älteren Literatur häufiger angegebenen grossen Entfernungen beruhen wahrscheinlich auf Beobachtungsfehlern. Es ist bekannt, dass die Wanderungsgeschwindigkeit und auch die zurückgelegten Entfernungen durch eine Reihe von Faktoren beeinflusst werden.

In neuerer Zeit hat WALLACE (1955, 1956, 1958a, 1958b) dieses Problem intensiv bearbeitet und besonders auf die Bedeutung der Wasserverhältnisse im Boden hingewiesen. In den vorliegenden Experimenten wurden hauptsächlich Fragen der chemischen Beeinflussung und der Beeinflussung durch Strömung untersucht.

Dass Nematoden überhaupt auf die Strömungsrichtung von Flüssigkeiten reagieren können, hat FÜLLEBORN (1924) bei Warmblüterparasiten gezeigt. Die von ihm untersuchten Larven von *Strongyloides* und *Ankylostoma* wanderten deutlich gegen die Strömung. BAUNACKE (1922) führt einen Versuch an, der auf ein ähnliches Verhalten bei Larven des Rübennematoden hindeutet.

Das Vorhandensein einer Rheotaxis wurde von uns in Versuchen nach Schema B (Abb. 6) geprüft. Durch die Verdunstung entsteht hier in der Bahn ein einseitiger kapillarer Strom, dessen Stärke von der Höhe der Verdunstung abhängt. Genaue Messungen konnten nicht durchgeführt werden, doch gaben die Abnahme der Flüssigkeit in den Schälchen und die Anreicherung bzw. Auskristallisation gelöster Stoffe am Ende der Bahn (Verdunstungsstelle) gewisse Anhaltspunkte.

Es stellte sich heraus, dass die hier untersuchten pflanzenparasitären Nematoden wesentlich schwächer auf Strömungen reagieren als die oben erwähnten Humanparasiten. Die Stockälchen verteilten sich im Filterpapier und im Sand, ohne dass ein Zusammenhang mit der Stromrichtung erkennbar gewesen wäre. Es scheint, dass die Wanderungsrichtung bei diesen Älchen von so kleinen Strömungen nicht beeinflusst wird.

Bei den *Heterodera*-Larven bestand eine deutliche Neigung, *mit* der Strömung zu wandern. In 25 Versuchen mit *H. rostochiensis*, in denen insgesamt rund 10 000 Tiere ausgezählt wurden, wanderten in 17 Fällen mehr als 65% der Älchen *mit* der Strömung und in 4 Fällen mehr als

65% *gegen* die Strömung. In den restlichen 4 Versuchen waren keine gesicherten Unterschiede nachzuweisen.

Es ist nicht ausgeschlossen, dass bei der Wanderung *mit* der Strömung auch ein gewisser passiver Transport stattfand. Wenigstens hat WALLACE (1959) bei senkrecht von oben nach unten fliessendem Strom eine Verbreitung der Nematoden, soweit sie sich bewegten, nach unten festgestellt. Da in den vorliegenden Versuchen aber verhältnismässig schwache und noch dazu horizontal verlaufende Strömungen vorhanden waren, dürfte dieser Faktor kaum eine Rolle gespielt haben, worauf ja auch die eindeutig *gegen* den Strom gewanderten Nematoden hinweisen.

Interessant ist, dass die Versuche mit Filterpapier hier im allgemeinen klarere Ergebnisse erbrachten als die Versuche in Sand. Das beruht wahrscheinlich darauf, dass in dem flachen, langfaserigen Substrat, vor allem zwischen Papier und Unterlage, eine gleichmässigere Strömung zustandekommt als in dem körnigen Sand. Ob der Strömung beim Verhalten der Larven im freien Feld eine grössere Bedeutung zukommt, lässt sich auf Grund dieser Versuche noch nicht entscheiden. Die Möglichkeit ist jedenfalls gegeben, da die Tiere auf Strömungen reagieren können.

Bei der Prüfung der Reaktionen auf chemische Reize, d.h. auf Wurzelsekrete, waren, wie aus den oben angeführten Beobachtungen hervorgeht, zwei Fragen zu unterscheiden, nämlich die Frage nach einer un-

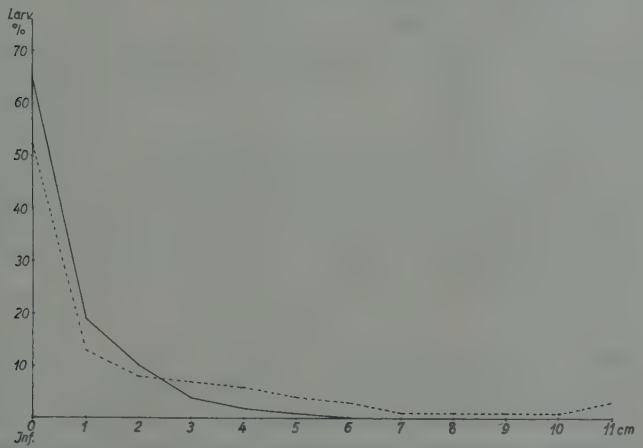

Abb. 8. Die Wirkung von Wurzelsekreten auf die Ausbreitung von Larven des Kartoffelnematoden. Auswertung erfolgte nach 14 Tagen. ---- = Sand mit Ablaufwasser befeuchtet; — = Sand mit Leitungswasser befeuchtet.

gerichteten allgemeinen Aktivierung der Larven und die Frage nach einer gerichteten Anlockung. Auf die erste Frage konnten Versuche nach Schema A und B (Abb. 6) Auskunft geben. Eine allgemein aktivierende Eigenschaft der Wurzelsekrete würde bewirken, dass sich die Larven in einer Bahn mit Ablaufwasser schneller und vielleicht auch weiter bewegen als in einer Bahn mit Wasser. Tatsächlich konnte beim Kartoffelnematoden ein solches Verhalten einwandfrei nachgewiesen werden. In den Ablaufwasserbahnen entfernten sich mehr Larven von der Infektionsstelle und waren nach einer bestimmten Zeit merklich weiter gewandert als die Älchen in den Wasserbahnen. Die Abb. 8 zeigt dafür ein Beispiel.

Man sieht zunächst einmal die schon erwähnte Tatsache, dass auch noch nach 14 Tagen mehr als die Hälfte der aufgetropften Larven (insgesamt etwa 14 000) innerhalb des 2 cm langen Abschnittes der Infektionsstelle blieb. Diese Erscheinung, die durchweg in allen Versuchen beobachtet wurde, demonstriert die verhältnismässig geringe Beweglichkeit der *Heterodera*-Larven. Trotzdem wird die anregende Wirkung der Wurzelsekrete dadurch deutlich, dass sich im Wasser nur 36% der aufgetropften Larven von der Ausgangsstelle entfernt hatten, im Ablaufwasser dagegen 48%. Dazu kommt noch, dass beim Abbruch der Versuche die ersten Larven im Wasserstreifen erst 6 cm weit gewandert waren, während die Larven im Ablaufwasserstreifen sich schon in grösserer Zahl am Ende der Bahn (11 cm) angesammelt hatten und bei einer längeren Bahn vermutlich noch merklich weiter gewandert wären. Damit darf angenommen werden, dass die Wurzelsekrete auf die freibeweglichen Larven eine aktivierende Wirkung ausüben.

Eine grosse Anzahl von Versuchen wurde dem Nachweis einer anlockenden Wirkung der Wurzelsekrete gewidmet. Trotzdem konnte mit den beschriebenen Methoden kein eindeutiges Ergebnis erzielt werden. Das Verhalten der Larven war meist indifferent. Durch Variation der Versuchsbedingungen konnte nachgewiesen werden, dass nicht methodische Fehler die Ursache dieser Unklarheit waren. Es muss daher angenommen werden, dass das Wurzelablaufwasser keine anlockende Wirkung hat.

Auf der anderen Seite zwingen Befunde, wie sie z.B. von Linford (1939), Wallace (1958c) und Widdowson, Doncaster & Fenwick (1958) erzielt wurden, zu der Annahme, dass von wachsenden Wurzeln eine chemische Anlockung der Nematoden ausgeht. Der Widerspruch ist wahrscheinlich dadurch zu erklären, dass in dem Ablaufwasser die

Konzentration der anlockenden Stoffe zu gering ist. Dafür spricht, dass auch in den oben zitierten Arbeiten eine Anlockung nur in unmittelbarer Nähe der Wurzeln beobachtet wurde. Damit müssen aber alle Angaben über eine Fernwirkung auf falschen Beobachtungen bzw. Interpretationen beruhen.

Nach den hier vorliegenden Versuchen wandern die Larven nach dem Schlüpfen zunächst ungerichtet. Sie werden durch die Wurzelsekrete nur zu einer schnelleren Fortbewegung angeregt. Die biologische Bedeutung dieses Verhaltens liegt wohl darin, dass so die Wahrscheinlichkeit, eine geeignete Wurzel zu finden, erhöht wird. Erst da, wo die Konzentration der wirksamen Stoffe genügend hoch ist, also in Wurzelnähe, erfolgt dann ein gerichtetes Wandern.

SUMMARY

Three groups of experiments were carried out to test the migratory behaviour of some plant parasitic nematodes.

1. Host plants were grown in sterilised soil and cysts of *Heterodera rostochiensis* and *H. schachtii* added at different distances from the plants. It was shown that larvae hatched only when roots came close (about 1-2 cm) to the cysts. No hatching or attraction were observed over appreciable distances. Usually larvae moved about 20 cm only during the growing season.
2. Cysts or larvae were placed in wooden boxes filled with sterilised soil without plants. Water or root diffusate was dropped onto the soil at various distances from the site of inoculation. Here also root diffusates had no influence on the larvae over a distance of more than 2-3 cm. However when the eelworms came in direct contact with the root diffusate they moved faster and farther. Some results suggested a definite attraction.
3. Nematodes were put on filter paper strips and into plastic channels filled with sand of 200-500 μ particle size. The strips and channels were connected by wicks dipping into solid watch glasses containing water or root diffusate, so that the sand and filter paper were kept moistened with these fluids by capillarity. Larvae of *Heterodera* appeared to show a rheotaxis. Greater numbers moved with the stream of fluid. Stem eelworms showed no noticeable reaction to the direction of the current. When the media were moistened with root diffusate, the larvae of *Heterodera* migrated faster and farther than when moistened with water. An orientated attraction could not be observed.

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INVESTIGATIONS ON THE LIFE CYCLE, REPRODUCTION, FEEDING HABITS AND HOST RANGE OF *TYLENCHORHYNCHUS CLAYTONI* STEINER [1])

BY

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The tobacco stunt nematode, *Tylenchorhynchus claytoni*, is one of the more serious plant-parasitic nematodes in North Carolina. It was first described from specimens collected around and in roots of stunted tobacco plants (*Nicotiana tabacum* L.) (Steiner, 1937). This nematode was found in soil from 67% of 175 tobacco fields sampled in South Carolina (Graham, 1954). Besides reproducing on cotton and tobacco it also reduced the root systems and caused stunting of these plants in the greenhouse. *T. claytoni* severely reduced the root systems of hybrid corn breeding lines in greenhouse tests (Nelson, 1956). Several inbreds were resistant resulting in reduction of the nematode populations. This species has been causing considerable damage to tobacco in North Carolina (Drolsom & Moore, 1955), and a recently developed variety (Dixie Bright 101) is reported to support larger nematode populations than old line varieties (Nusbaum, 1954). *T. claytoni* has been found in golf green turfs (Troll & Tarjan, 1954), in the soil around roots of sugar cane, strawberry, camellia, sweet potato and rice (Martin & Birchfield, 1955), and in the rhizospheres of chlorotic and stunted peanut plants (Boyle, 1950).

Host-parasite relations have been studied with two other *Tylenchorhynchus* species. The relations of *T. dubius* Bütschli to cotton and Tepary bean (*Phaseolus acutifolius*, Gray, var. *latifolius*, Freem.) were investigated in Arizona (Reynolds & Evans, 1953). Nematodes appeared to feed on the epidermal tissues of young roots and larvae were found inside some small secondary roots. The nematode reproduced upon and caused stunting of both hosts in the greenhouse, but did not live on several fungi tested in agar culture. Another species, *T. martini*

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Fielding, reproduced upon and reduced the root systems of sugar cane plants (Birchfield & Martin, 1956). On the basis of nematode reproduction, Johnson grass (*Sorghum halepense* (L.) Pers.) and rice were favorable host plants, soybean and sweet potato were fair hosts, and cotton, corn, sweet clover and Ligustrum were poor or non-hosts.

Since *T. claytoni* is an important crop pest and little has been reported concerning its biology, studies were undertaken on: 1) the life cycle, 2) the feeding habits, 3) the effect of temperature on reproduction, 4) the longevity in soil in the absence of plants, and 5) the host range.

MATERIALS AND METHODS

Stock cultures of *T. claytoni* were maintained on tobacco (Dixie Bright 101) in the greenhouse at 70-80° F. Nematodes for inoculation purposes were removed from the soil employing a combination of sieving and Baermann funnel techniques (Christie & Perry, 1951). Nematodes were collected from the funnels within 24 hours, stored at 5° C and used within 72 hours.

Plant material for host range and temperature studies was propagated from transplants or seeds. Young plants of tobacco, tomato, pepper, lettuce, wheat and sweet potato were used for transplanting, and "eyes" of Irish potatoes were planted directly. Seeds were either germinated on moist filter paper in Petri dishes before planting or seeded directly.

The sandy loam soil used in all tests was fumigated with methyl bromide. In addition, soil used in the studies on the role of males in reproduction was steamed for 4 hours in 3-in.-deep wooden flats. All pots were steam disinfested. Fertilizer was added to the soil in quantities sufficient for good plant growth.

Studies on the host range and reproduction were conducted in a greenhouse at 70-80° F. The temperature range and longevity studies were conducted at 45° F, 55° F, 65° F, 75° F, 70-80° F and 85-95° F. The first 4 temperatures were obtained in controlled temperature rooms with fluorescent light as the light source; the latter 2 temperatures were obtained in greenhouses.

A mixture of 200 males, females and larvae of *T. claytoni* in water suspension was added to each pot of soil used in the host range, temperature range and longevity studies immediately before planting. A pipette drawn from 7 mm glass tubing with a rubber tube attached to the enlarged end was used for collecting and counting the nematodes. When suction was applied to the tube, nematodes in water suspension

entered the fine tip of the pipette and as they entered were counted with the aid of a stereoscopic microscope.

In the host range and temperature studies the nematodes were assayed as follows: the entire pot of soil, including soil washed from the plant roots, was stirred for 3-4 minutes with two portions of 4-5 quarts of water. The suspension was passed through a 20-mesh screen to remove coarse debris and the combined volume of suspension was made up to 10 quarts. One quart of the thoroughly mixed suspension was poured through a 325-mesh screen and the residue was placed on submerged wet-strength tissue paper in a Baermann funnel (ANDERSON & YANAGIHARA, 1955). Nematodes were recovered similarly from the soil in the studies on reproduction and longevity, but the entire 10 quarts of suspension was passed through a 325-mesh screen. After 5 days samples were drawn from the funnels and nematode counts made.

The feeding habits and life cycle were studied by placing nematodes close to roots of alfalfa plants (Atlantic) growing in sterile nutrient agar medium, pH 5.4, in Petri plates, similar to that described by TORREY, 1954. The nematodes and alfalfa seeds were disinfested before being placed on the agar medium. The seeds were disinfested by exposure in a Petri plate for 48 hours to vapors from 3 drops of Panogen (21% methyl-mercury-dicyandiamide) placed on a piece of folded filter paper. Then two seeds were transferred aseptically to each Petri plate of medium. Nematodes were disinfested by subjecting them to two 15-minute baths in 3700 ppm. streptomycin sulfate solution (filtered 1% Agrimycin, which contains 37% streptomycin sulfate). Then the nematodes were transferred directly with a sterile needle to the agar medium just above the alfalfa roots growing through the medium. The roots were 2-5 cm long at the time of nematode inoculation. Alfalfa was used as the host plant because it grew slowly and a seedling could be maintained within a 4.5 cm Petri plate for a month without crowding. The nematode-inoculated plates were placed in plastic bags under fluorescent light at 75° F. and the nematodes examined microscopically each day.

Small blocks of agar containing roots upon which nematodes were feeding were removed from the Petri plates for histological examination. The agar blocks were fixed in CRAF III (30 ml 1% chromic acid, 20 ml 10% acetic acid, 10 ml 40% Formalin, 40 ml water) (SASS, 1951), dehydrated in an ethanol-xylene series, and embedded in Tissuemat (Fisher, M. P. 55-58° C.). Sections were cut 10 microns in thickness and stained with safranin and fast green.

The general methods used are explained above. Specific methods for a particular study are given in the experimental results with that study.

EXPERIMENTAL RESULTS

Studies on the Life Cycle

The time required to complete the life cycle of *T. claytoni* was studied by placing a single fertilized female on the agar medium in each of 150 4.5 cm Petri plates each containing an alfalfa seedling. Nematode development was followed by daily microscopic examination. The complete life cycle from egg to egg was not obtained in these cultures, but the cycle from egg to adult was followed. About fifty of the females laid from 1 to 15 eggs each; 124 viable laid by thirty-four females hatched in an average of 5.9 days with a standard error of ± 0.4 days. Egg-laying continued for approximately 1 week and eggs were always deposited close to alfalfa roots where the nematodes fed. Many eggs were laid near the root caps so that on hatching the larvae were close to the root hairs. Most of the larvae failed to develop beyond the 2nd or 3rd stage, but in one plate two mature males were found after 33 days. This indicates that *T. claytoni* in monoxenic culture on alfalfa seedlings growing on nutrient agar medium completes its life cycle in about 1 month. Very little fungal or bacterial contamination was experienced in these cultures. Most of the alfalfa seedlings were still growing at the end of the experiment.

The study of the role of males in reproduction was set up as follows: one hundred 6-in. pots of soil were infested with one nematode larva per pot, fifty pots received two larvae each, and twenty five pots received one male and one female per pot. Immediately after infestation a seedling of tobacco (Dixie Bright 101) was planted in each pot. Plants were grown for 70 days in the greenhouse at 70-80° F after which the soil was screened for nematodes.

No reproduction occurred in any of the pots infested with a single larva; 11 nematodes, 5 males, 5 females and 1 larva, all from 11 separate pots, were recovered by screening. Reproduction occurred in only one of the fifty pots receiving two larvae each and this culture yielded 3 males, 5 females and 14 larvae. From ten other separate pots in the same group 3 males, 6 females and 1 larva were recovered as solitary individuals. Population increase occurred in eighteen of the twenty five pots infested with one male and one female per pot. These populations were highly variable in numbers and the largest nematode population yielded 30 males, 129 females and 124 larvae.

Females recovered from the pots infested with two larvae per pot were examined microscopically. Four of the five females from the one pot in which reproduction took place contained sperms in the spermathecae indicating that copulation had occurred; the 5th female lacked sperm in the spermatheca. None of the six females from the separate pots where no reproduction took place had sperms in their spermathecae.

On the basis of these results it is concluded that males are necessary for the reproduction of *T. claytoni.*

Feeding Habits

With the aid of a steroscopic microscope, specimens of *T. claytoni* were observed feeding on alfalfa roots growing in a nutrient agar medium in Petri plates. More critical observations on feeding habits were made with a compound microscope. A block of agar containing roots and nematodes was transferred to a glass slide. A cover slip was so placed on the agar block that nematodes feeding on the root could be seen. Observation on single nematodes lasted for an hour or more and the feeding habits of about fifteen nematodes were studied.

Nematodes were seen feeding only on epidermal cells in the region of cell elongation and between the root hairs. Most nematodes fed on epidermal cells among the root hairs, but none were observed feeding on the root hairs. Nematodes often became completely embedded in the root cap cell mass, but no feeding was seen on those cells. Feeding on older portions of the roots, or on stems or leaves which became submerged in the agar, was not observed.

In the search for a feeding area the nematode glided over the root surface with the stylet angled towards the plant epidermis. As the nematode moved tentative jabs were made by the stylet at various epidermal cells. When a spot favorable for feeding was found, a series of rapid jabs was made with the stylet until the epidermal cell wall was punctured. During feeding the nematode body generally lay longitudinally or transversely close to the root surface with the anterior portion raised above the root allowing the lip region and stylet to be projected towards the epidermal cell and the lips were pressed against the plant cell (Fig. 1). The stylet was protruded one-fifth of its length from the nematode body and the median esophageal bulb pulsated rapidly. Feeding sometimes continued for one-half hour in the same spot.

No histological evidence of nematode feeding on root epidermal cells was obtained. There was no indication by staining reactions or punctures that feeding or injury had occurred to any root cells. Although

no histological evidence of nematode injury to roots was observed root systems of tobacco (Dixie Bright 101) growing in pots were considerably reduced by large populations of *T. claytoni* (Fig. 2).

Effect of Temperature on Reproduction

Tobacco was tested at temperatures ranging from 55° F to 85-95° F and wheat from 35° F to 85-95° F. In addition to the nematode population counts, only the general nature of plant growth after 90 days at the various temperatures was recorded. Since wide variations in the nematode populations occurred between temperatures, the data were transformed to logarithms for the statistical analysis to stabilize the experimental error.

TABLE I

Effect of temperature on the reproduction of Tylenchorhynchus claytoni *on tobacco and wheat*

Temp. °F.	Test 1[a]		Test 2		Test 3		Overall Mean[c]	Test 4		Test 5	
	A[b]	B	A	B	A	B	(Log.)	A	B	A	B
35								38	1.552	16	0.981
55	238	2.351	38	1.400	12	0.581	1.444	—	—	12	0.756
65	330	2.310	2	0.200	280	2.411	1.640	362	2.484	4	0.400
75	8,544	3.852	7,168	3.781	8,246	3.688	3.774	762	2.819	206	2.147
70—80	912	2.854	2,752	3.368	1,602	3.112	3.112	7,194	3.780	1,624	3.033
85—95	12,958	4.042	13,600	4.035	15.138	3.855	3.977	1,344	3.026	640	2.618
Test Means		3.082		2.557		2.729	2.788				
L.S.D. .05									0.369		0.754

L.S.D. s for Tests 1, 2 and 3. (Log data)

L.S.D. .05 for Temperature Means = 0.314

L.S.D. .05 for Test Means = 0.244

L.S.D. .05 for Temperature-Variety Interaction = 0.546

[a] The host plants were: Test 1, Virginia Gold tobacco; Tests 2 and 3, Dixie Bright 101 tobacco; and Tests 4 and 5, Atlas 66 wheat. The tobacco data, Tests 1, 2 and 3, were combined for analysis and the data for wheat, Tests 4 and 5 were analyzed separately.

[b] A is the arithmetic mean and B the logarithmic mean. The data were analyzed from the logarithmic means.

[c] The overall logarithmic means for the tobacco data, Tests 1, 2 and 3.

T. claytoni reproduced over a relatively wide range of temperatures (Table I). There was little reproduction on tobacco at 55° F or 65° F,

L. R. KRUSBERG: *Investigations on* Tylenchorhynchus claytoni.

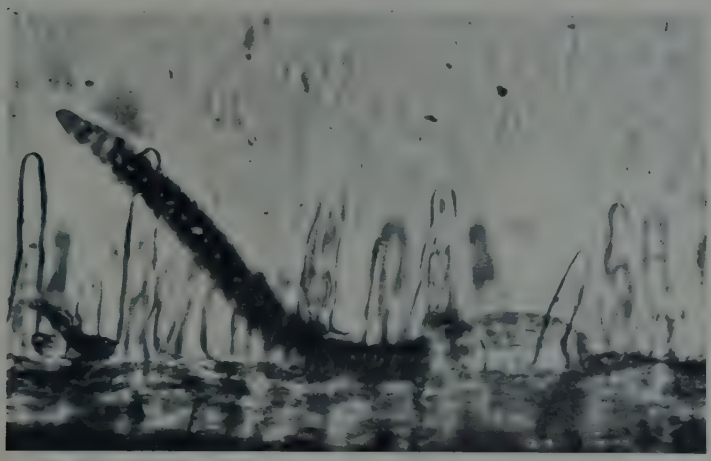

Fig. 1. A larva of *T. claytoni* feeding on an epidermal cell of an alfalfa root growing in sterile nutrient agar medium. (X 400).

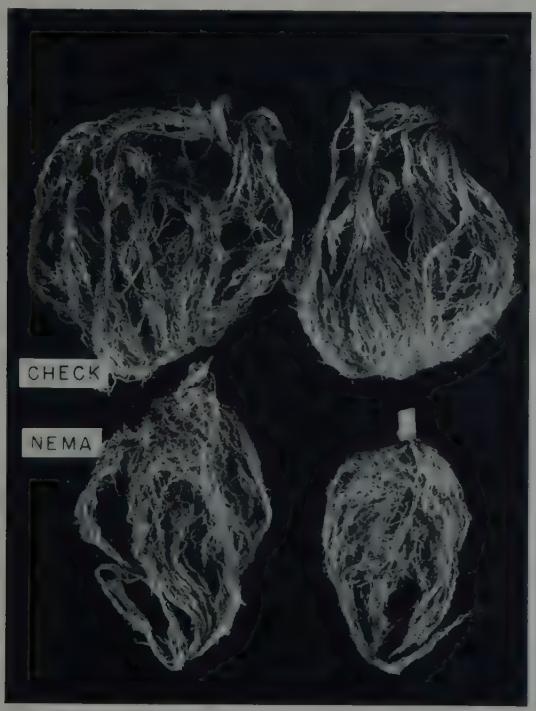

Fig. 2. Comparison of root systems of tobacco plants (Dixie Bright 101) grown in soils free of and heavily infested with *T. claytoni*.

but the number of nematodes increased significantly at 75° F. Although the populations were not significantly different from those at 75° F, the largest mean numbers of nematodes were recovered at 85-95° F. The nematode populations at 70-80° F were lower than those at 75° F or 85-95° F. On wheat the largest nematode populations were present on plants at 70-80° F. The populations at this temperature were significantly different from those at 75° F and 85-95° F in Test 4, but not Test 5. At 35° F and 55° F there was no reproduction. Both tobacco and wheat grew best at 70-80° F.

Longevity in the Soil in the Absence of Plants

Nematodes were placed in soil that was kept moist by periodic watering and kept free of plants. Soil from 1 of 10 4-in. pots maintained at each of six temperatures was screened at 30-day intervals on 10 consecutive occasions.

The number of specimens of *T. claytoni* recovered was inversely related to both time and temperature. Regression coefficients for time and temperature were significantly different from zero and showed an average decrease of 4.2 nematodes per pot for each 30-day period and a decrease of 3.5 nematodes per pot for every increase of 5° F starting at 35° F. Nematodes were still recovered at the end of 10 months at temperatures of 35° F through 75° F. Nematodes obtained from pots after several months at higher temperatures were conspicuously devoid of body contents as compared to those from pots at lower temperatures which retained relatively normal appearance.

Host Range Studies

In these experiments forty-one plant varieties were tested for their suitability as hosts for *T. claytoni*. Plants were grown in five replicate 6-in. pots of nematode-infested and five of non-infested soil. After plants had grown for 90 days the plant tops were weighed and the nematode populations in the soil from the infested plants were determined. Plant root systems were not examined or weighed.

The plants under test were arbitrarily classified on the basis of nematode reproduction and divided into three groups as follows: 1) highly favorable for nematode reproduction (averaging 1000 or more nematodes per pot) 2) favorable for nematode reproduction (averaging 200-1000 nematodes per pot) and 3) unfavorable for nematode reproduction (averaging less than 200 nematodes per pot) (Table II). Plants in groups 1 and 2 were tested once and those in group 3 at least twice.

TABLE II

Reproduction of Tylenchorhynchus claytoni *in host range tests*

Scientific Name	Common Name	Variety	Mean ± Stan. Error of the Mean
1. Plants highly favorable for reproduction.			
Nicotiana tabacum L.	tobacco	Bottom Special	16,554 ± 2,047
	tobacco	Coker 139	8,632 ± 3,063
	tobacco	Dixie Bright 101	3,594 ± 970
Sorghum vulgare var. *sudanese* (Piper) Hitchc.	sudan grass	Sweet	5,682 ± 923
Solanum tuberosum L.	Irish potato	Irish Cobbler	5,364 ± 2,449
	Irish potato	Kennebec	4,696 ± 772
Triticum aestivum L.	wheat	Atlas 66	4,424 ± 836
Zea mays L.	corn	Dixie 18	4,350 ± 1,397
	corn	Ioana	3,702 ± 882
	corn	N. C. 27	1,386 ± 151
Sorghum vulgare (Pers.) Martin	milo	Martin	3,242 ± 522
Phaseolus vulgaris L.	bean	Contender	3,042 ± 882
Trifolium pratense L.	red clover	Common	2,416 ± 811
Xanthium echinatum Murr.	cockle burr		2,356 ± 843
Vicia villosa Roth	vetch	Common Hairy	2,274 ± 959
Hordeum vulgare L.	barley	Colonial	2,236 ± 384
Avena sativa L.	oats	Arlington	1,750 ± 288
	oats	Fullgrain '52	1,450 ± 257
	oats	Victorgrain 48-93	1,434 ± 492
Secale cereale L.	rye	Abruzzi	1,424 ± 586
Ipomoea batatas Poir.	sweet potato	Porto Rico	1,710 ± 632
Festuca elatior L.	fescue	Kentucky 31	1,416 ± 471
Lactuca sativa L.	lettuce	Leaf	1,304 ± 404
Lespedeza cuneata (Dumont) G. Don	lespedeza	Sericea	1,120 ± 209
Lespedeza stipulacea Maxim.	lespedeza	Korean	1,092 ± 346
2. Plants favorable for reproduction.			
Gossypium hirsutum L.	cotton	Coker 100 MR	942 ± 225
Lycopersicon esculentum Mill.	tomato	Rutger's	936 ± 230
Trifolium repens L.	clover	White Dutch	870 ± 340
Trifolium incarnatum L.	clover	Crimson	608 ± 124
Setaria italica (L.) Beauv.	pearl millet	Common	514 ± 87
Phaseolus vulgaris L.	peas	Sugar Crowder	490 ± 344
Glycine max (L.) Merr.	soybean	Ogden	476 ± 145
Medicago sativa L.	alfalfa	Atlantic	464 ± 145
	alfalfa	Kansas Common	382 ± 185
Dactylis glomerata L.	orchard grass	Common	434 ± 70
Trifolium pratense L.	red clover	Kenland	302 ± 90
Lespedeza stipulacea Maxim.	lespedeza	Rowan	274 ± 90
Trifolium repens L.	clover	Ladino	216 ± 75
3. Plants unfavorable for reproduction.			
Capsicum frutescens (L.) Bailey	pepper	California Wonder	48 ± 22
Cucumis sativus L.	cucumber	Model	18 ± 7
Crotalaria spectabilis Roth	showy crotalaria		10 ± 8
Arachis hypogaea L.	peanut	N. C. 2	5 ± 3

Tobacco, particularly Bottom Special variety, was the most favorable host for the reproduction of *T. claytoni*. Other plants also highly favorable for reproduction were corn, sudan grass, small grains (wheat, oats, barley, rye, gram), Irish potato, sweet potato, milo and several legumes. Plants favorable for nematode reproduction were soybean, alfalfa, lespedeza, several clovers, cotton, tomato, orchard grass and pearl millet. Pepper, cucumber, peanut and showy crotalaria were unfavorable for nematode reproduction — in most pots less than 100 nematodes were recovered. The top weights of the plants were highly variable and there was no correlation of the top weights with nematode populations. Frequently, the tops of plants supporting large nematode populations were heavier than those of the control plants growing in non-infested soil.

DISCUSSION

Plant growth in the host range studies was not related to nematode populations. In fact, in some instances top weights of plants supporting large populations of *T. claytoni* were larger than those of nematode-free check plants. With initial infestations of 200 nematodes per pot, plants favorable for reproduction completed most of their growth before the populations reached damaging proportions and thereby escaped serious injury.

Nematode population build-up may not be a good basis for classifying plants as to favorability to nematode reproduction. The root systems of plants vary greatly in size. For instance, tobacco has a larger root system than red clover. The size of the root system may influence the amount of nematode reproduction.

The lack of or low rate of reproduction on favorable plants, such as, small grains, grown below 65° F indicates that during winter population increases may be insignificant on these plants. However, nematodes may persist in the soil even in the absence of host plants for several months at low temperatures (35-75° F). As the temperature increases above 65° F the reproductive rate increases. Nematode reproduction may also vary directly with the optimum temperature for plant growth. The greatest reproduction on wheat was at 70-80° F, on tobacco at 85-95° F.

The use of whole plants and plant tissues growing in aseptic culture on a nutrient agar medium is advantageous for many studies with plant parasitic nematodes, such as, observations on feeding, life cycle studies, and evaluation of damage caused to plants by nematodes in the absence

of other microorganisms. On the other hand, artificial culture has certain disadvantages, such as, both the plant and nematode are considerably separated from their natural environment which may result in poor reproduction and growth of the nematodes; furthermore, nematode damage to plant roots in the presence of the soil microflora cannot be estimated. The parasitic complex involving nematodes propagated on plant tissues in monoxenic culture cannot with certainty be considered as identical with the plant-nematode complex in the soil environment; however, they should be similar.

Although *T. claytoni* has been reported as endoparasitic in plant roots (Steiner, 1937), only ectoparasitic feeding on the epidermal cells of alfalfa root tips was observed in these studies. However, some specimens did penetrate the loose peripheral cell layers of the root cap. There was no evidence of injury to the root or root cap cells.

ZUSAMMENFASSUNG

Untersuchungen über den Wirtspflanzenkreis von *Tylenchorhynchus claytoni* zeigten, dass vier von einundvierzig Pflanzenarten oder Varietäten, nämlich Erdnüsse (N.C. 2), Pfeffer (California Wonder), Gurke (Model) und *Crotalaria spectabilis,* die Vermehrung des Nematoden ungünstig beeinflussten. *T. claytoni* vermehrte sich am schnellsten bei einer Temperatur von 85-95° F an Tabak (Dixie Bright 101) und bei einer Temperatur von 70-80° F an Weizen (Atlas 66). Untersuchungen über die Langlebigkeit der Art wurden mit je 200 Nematoden in feuchter Erde ohne Pflanzen ausgeführt. Es ergab sich ein Verlust von 4.2 Nematoden für jede 30-Tag Periode und ein Verlust von 3.5 Nematoden je 5° F Temperaturerhöhung beginnend mit 35° F. *T. claytoni* lebte 10 Monate lang in feuchter Erde ohne Pflanzen bei einer Temperatur von 35-75° F. Männchen sind für die Vermehrung der Art anscheinend notwendig. Die Nematoden ernähren sich an Luzernesämlingen von den Epidermiszellen der Wurzelspitze und der Wurzelhaarzone. Ein Eindringen der Nematoden trat nur in den peripheren Zellagen der Wurzelhaube ein. Die Nematodenlarven schlüpften 6 Tage nach Ablage der Eier und entwickelten sich in monoxenischer Kultur, d.h. unter Ausschluss anderer Organismen, and Luzernesämlingen auf Nähragarmedium in ungefähr 33 Tagen zur Geschlechtsreife.

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CHEILORHABDITIS AND *ODONTORHABDITIS*, TWO NEW GENERA OF SOIL NEMATODES ALLIED TO *RHABDITIS*

BY

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Large numbers of two closely-related genera of soil nematodes were obtained from a rotting underground stalk of cultivated banana (*Musa paradisiaca* L.) on the grounds of Notre Dame College, Dacca. Both genera strongly resembled the most common genus of soil nematodes, *Rhabditis*, but differed markedly in the structure of the stoma. Both genera are unique in the possession of a large dorsal tooth or transverse plate, developed from the wall of the meso-metarhabdions. The one genus has sclerotized, strongly curved cheilorhabdions. For this new genus the name *Cheilorhabditis* is proposed. The other genus has unsclerotized, slightly curved cheilorhabdions. For this new genus the name *Odontorhabditis* is proposed.

Both genera were observed in the living state and were found to display marked agility. Measurements of all specimens were made after death by gentle heat fixation. The measurements and the sketches of the type specimens, however, are of glycerine jelly mounts.

CHEILORHABDITIS N.G.

Diagnosis. Family Rhabditidae, Subfamily Rhabditinae. Head set off, lips fused at base; stoma consisting of separate cheilorhabdions, prorhabdions, meso-metarhabdions, and telorhabdions; cheilorhabdions six in number, sclerotized, curved in form of question mark; protostom straight, with parallel walls; meso-metarhabdions with transverse tooth or plate arising from dorsal wall; telostom inclined anteriorly on ventral side; esophagus consisting of long procorpus, swollen metacorpus, narrow isthmus, and terminal bulb containing valvular appartus; female reproductive system didelphic; ovaries reflexed; male reproductive system single; testis reflexed; equal cephalated spicules with ventral tooth; gubernaculum triangular; copulatory bursa mostly postanal, with nine pairs of genital papillae; female tail long, at first conical then gradually tapering; male tail conical-spicate.

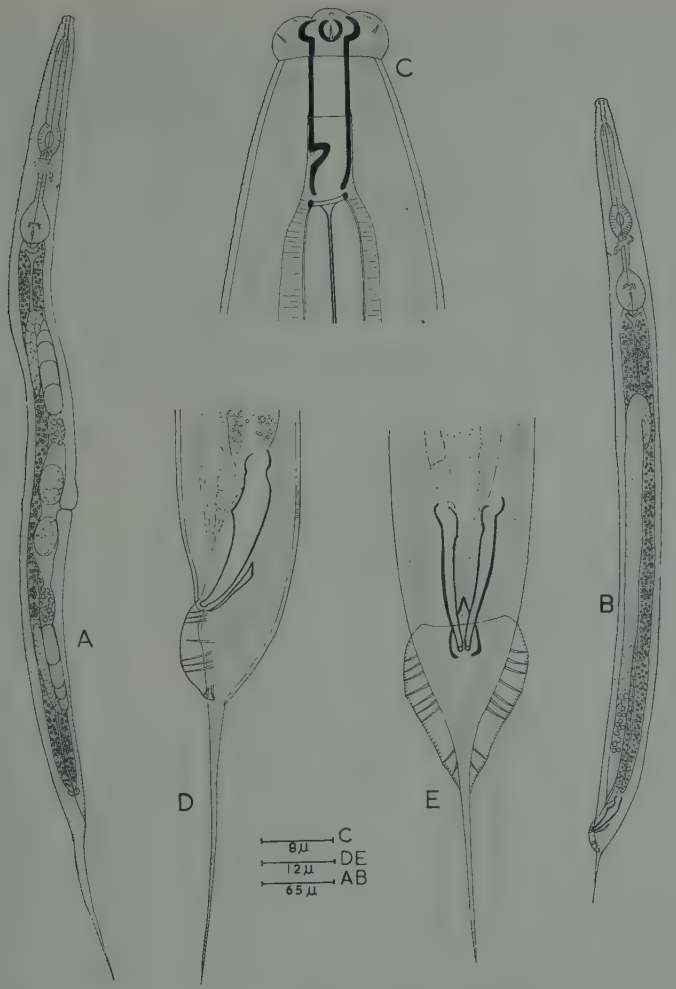

Fig. 1. *Cheilorhabditis dacchensis* n.g., n.sp. A. Entire female (ovaries shown on same side of intestine). B. Entire male. C. Female head, lateral view. D. Male tail, lateral view. E. Male tail, ventral view.

The name *Cheilorhabditis* is derived from χειλη lip, and ῥάβδος, rod, to which the suffix —ῖτις is added, making the name feminine in gender.

Type species. Cheilorhabditis dacchensis n. sp.

The genus differs from the other genera of the Rhabditinae in the presence of a conspicuous dorsal tooth or transverse plate. It differs from the new genus *Odontorhabditis* in the sclerotized cheilorhabdions of the stoma, curved in the form of a question mark.

CHEILORHABDITIS DACCHENSIS N. SP.

Fig. 1, A-E

10 ♀♀: 0.77-1.2 mm. (9.3); a, 19.1-24; b, 4.2-4.7; c, 4.5-7; V, 50-51.7%; Ov_1, 24.7-27%; Ov_2, 22.2-25.2%.

10 ♂♂: 0.61-0.74 mm. (0.68); a, 19-29.7; b, 3.6-4.3; c, 7.4-11.6.

Holotype ♀: L. 892 μ; a, 24; b, 4.6; c, 7.3; V, 51.5%, ova 1 + 1; tail 5.3 anal diameters.

Allotype ♂: L. 702 μ; a, 22; b, 4; c, 10.6; spicules 32 μ; tail 4 anal diameters.

Description: Body tapering slightly toward anterior and toward anal region. Cuticle double layered, the outer layer separating slightly after evaporation to glycerine. Exceedingly close transverse striation, usually inconspicuous but clearly punctate in decomposing specimens. Fine longitudinal fibrils in inner layer of cuticle. Lateral fields not differentiated. Head offset in form of rounded cap. Six lips, fused at base. Inner circle of 6 labial papillae; outer circle of 6 fine setose papillae and possibly 6 tiny papillae. Amphids small, papilloid, somewhat dorsally situated on lateral lips. Stoma 18-19 × 4-4.5 μ. Cheilostom well-developed, with 6 sclerotized cheilorhabdions curved in form of question mark. Prorhabdions well set off from cheilorhabdions and meso-metarhabdions. Walls of meso-metastom nearly parallel. Large blunty-rounded tooth arising from dorsal wall toward base of meso-metastom, appearing as transverse plate or shelf when seen in ventral view. Telostom inclined slightly toward anterior on ventral side. Esophagus divided into elongate procorpus, swollen metacorpus, narrow isthmus, and valvulate terminal bulb, followed by a short hemispherical esophageal-intestinal valve. Nerve ring crossing esophagus just below metacorpus. Excretory pore just below nerve ring. Intestinal cells distinct, dark-colored due to olivaceous spheroids.

Female: Female reproductive system didelphic; ovaries on opposite sides of intestine, reflexed about halfway to vulva; oocytes in double row the full length of the ovary; few oocytes in oviduct. One mature

ovum in each uterus at a time; ova in many-cell stage before deposition, 41-45 × 23-26 μ. Sperm in hind part of uterus, separated by slight constriction as seminal receptacle. Tail about 9 anal diameters long, conical the first 1/6, then gradually tapering to fine tip.

Male: Male reproductive system single; testis mostly ventral to intestine, reflexed a short distance. Copulatory bursa small, beginning just anterior to anus and enveloping conical part of tail. Nine pairs of genital papillae, grouped according to pattern: 3-1-3-2. Paris 1, 2, 3, and 5, 6, 7 turn slightly inward, while pairs 4 and 8 turn decidedly outward. Pair 8 represent phasmids. Copulatory spicules distinctly cephalated, with large ventral tooth at mid-length 29-32 μ long. Gubernaculum curved to fit spicules, triangular in shape with broad base and joining distal tips, 17-19 μ long. Tail 1/5 to 1/7 conical, 4/5 to 6/7 spicate, about 4 anal diameters long.

Holotype female. — Slide No. S 3, personal collection.

Allotype male. — Slide No. S 4, personal collection.

Paratypes (male and female). — Slide Nos. S 5 and S 6.

Type habitat. — Rotting stalk of *Musa paradisiaca* L. (cultivated banana).

Type locality. — Notre Dame College, Dacca, East Pakistan.

ODONTORHABDITIS N.G.

Diagnosis. Family Rhabditidae, Subfamily Rhabditinae. Head set off, lips arched and fused at base; stoma consisting of separate cheilorhabdions, prorhabdions, meso-metarhabdions, and telorhabdions; cheilorhabdions slightly curved, unsclerotized; prostom straight with parallel walls; ventral wall of meso-metastom curved inward at base; transverse dorsal tooth or plate arising from dorsal wall; telostom inclined anteriorly on ventral side; esophagus with long procorpus, swollen metacorpus, narrow isthmus, and terminal bulb containing valvular apparatus; female reproductive system didelphic, ovaries reflexed; male reproductive system single, testis reflexed; equal cephalated spicules with ventral tooth; gubernaculum triangular; copulatory bursa with nine pairs genital papillae; male and female tails both conical-spicate.

The name *Odontorhabditis* is derived from the combining form ὀδούς, ὀδοντ — tooth, and ῥάβδος rod, to which the suffix — ῖτις is added, making the name feminine in gender.

Type species. — *Odontorhabditis musicola* n. sp.

The genus is most closely related to the new genus *Cheilorhabditis*.

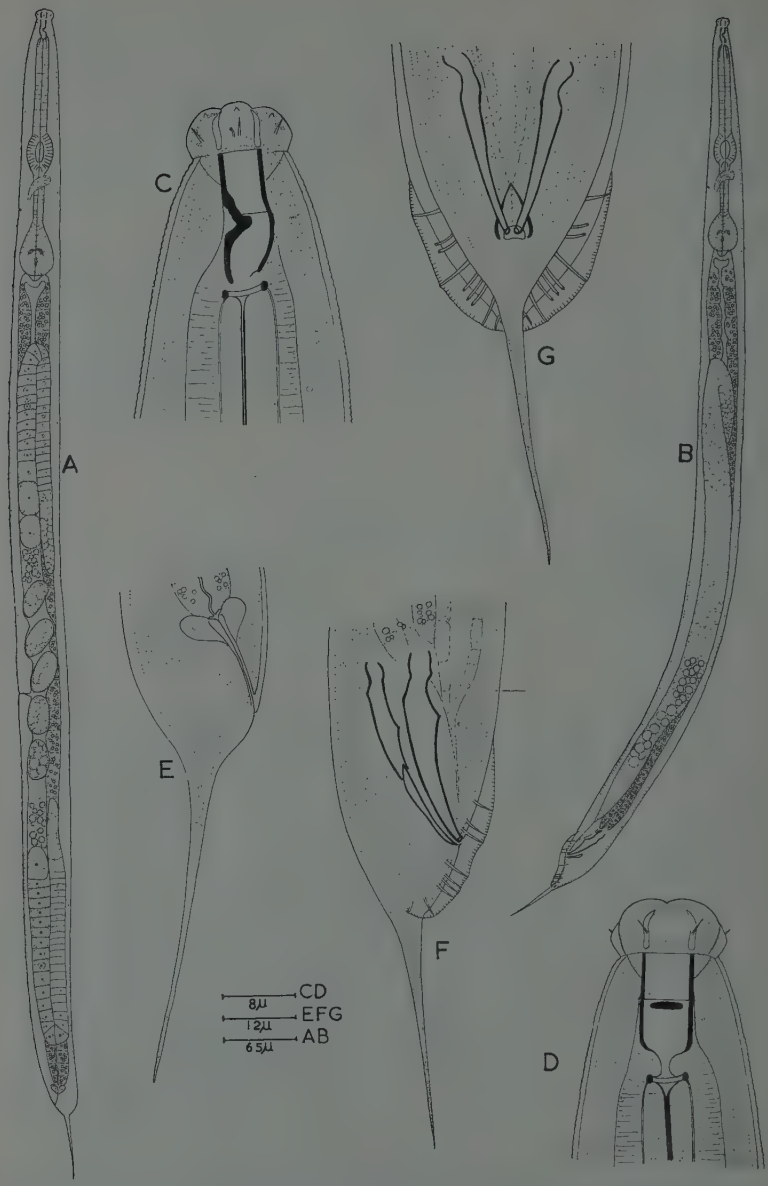

Fig. 2. *Odontorhabditis musicola* n.g., n.sp. A. Entire female (ovaries shown on same side of intestine). B. Entire male. C. Male head, lateral view. D. Male head, ventral view. E. Female tail. F. Male tail, lateral view. G. Male tail, ventral view.

It differs from it in the unsclerotized cheilorhabdions, not curved in the form of a question mark; in the greater proportion of breadth to width of the stoma; in the more anterior position of the dorsal tooth; in the curved ventral wall of the meso-metastom; in the more anterior extension of the bursa; and in the absence of dimorphism of the tails.

ODONTORHABDITIS MUSICOLA N. SP.

Fig. 2

10 ♀♀: 0.8-1.08 mm. (1.01); a, 16-21.5; b, 3.8-4.5; c, 11.2-15.4; V, 54.2-57.5%; Ov_1, 25-30%; Ov_2, 22-30%.

10 ♂♂: 0.64-1.05 mm. (0.85); a, 15-24.7; b, 3.3-4.5; c, 8.2-14.4.

Holotype ♀: L. 834 μ; a, 18.5; b, 4.3; c, 13; V, 55%; ova 1 + 1; tail 4 anal diameters.

Allotype ♂: L. 620 μ; a, 20; b, 3; c, 10; spicules 38 μ; tail 3 anal diameters.

Description. Appearance of body and cuticle as in previous species. Head offset, slightly retractible; cap less rounded at base than in previous species; 6 lips, fused at base. Inner circle of 6 small labial papillae; outer circle of 6 fine setose papillae and possibly 6 tiny papillae. Amphids papilloid. Stoma 19-22 × 7.5 μ at widest part. Cheilorhabdions unsclerotized, slightly curved inward. Prorhabdions well set off from cheilorhabdions and meso-metarhabdions. Ventral wall of meso-metastom curved inward at base; dorsal wall bearing large transverse dorsal tooth at anterior margin of meso-metastom, seen from ventral side as shelf or plate. Telostom inclined slightly toward anterior on ventral side. Esophagus, nerve ring, and excretory pore as in previous species.

Female. Female reproductive system didelphic; ovaries on opposite sides of intestine, reflexed almost to vulva; many oocytes in single row in oviduct; oocytes in double row only in posterior 1/3 of ovary. Up to 6 mature ova in uteri at a time, 45-51 × 25-29 μ; ova in many-cell stage or embryonated before deposition. Sperm in hind part of uterus, not separated by constriction as a receptacle. Tail conical-spicate; spicate portion 1/5-1/7.

Male. Male reproductive system as in previous species. Bursa extending more anteriorly and first pair genital papillae separated from pairs 2 and 3; pattern: 1-2-1-3-2. Large spherical sperm in vas deferens, conspicuous. Spicules cephalated, 38-45 μ long, with ventral tooth. Gubernaculum triangular with broad base and distal tips joined, 18-19 μ long. Tail conical-spicate, spicate portion 1/4-1/5.

Holotype female. Slide No. S 7, personal collection
Allotype male. Slide No. S 8, personal collection.
Paratypes (male and female). — Slide Nos. S 9 and S 10.
Type habitat. Rotting stalk of *Musa paradisiaca* L. (cultivated banana).
Type locality. Notre Dame College, Dacca.

Acknowledgement. The author wishes to thank Dr. I Andrássy for confirming the diagnosis as new of the genera reported in this paper, on the basis of rough sketches sent to him.

RÉSUMÉ

Cheilorhabditis *et* Odontorhabditis, *deux nouveaux genres de nématodes du sol apparentés au genre* Rhabditis

L'auteur donne la diagnose de deux nouveaux genres de nématodes libres du sol apparentés à *Rhabditis*. Chaque genre est représenté par une espèce dont l'auteur donne la description du mâle et de la femelle. Les deux genres se différencient de *Rhabditis* par la présence d'une dent dorsale ou plaque transversale formée par la paroi des meso-metarhabdions.

Le genre *Cheilorhabditis* est caracterisé principalement par la présence des cheilorhabdions sclerotisés, courbés en forme de point d'interrogation.

Le genre *Odontorhabditis* est caracterisé principalement par la présence des cheilorhabdions légèrement courbés mais non sclerotisés. Les deux genres ont été trouvés dans la partie souterraine d'un bananier pourri.

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CRICONEMOIDES AXESTE N. SP. ASSOCIATED WITH ROSES IN COMMERCIAL GREENHOUSES IN NEW YORK STATE

BY

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Collections of rose roots and soil from seven of eighteen commercial greenhouses in New York State yielded numerous specimens of a nematode belonging to the genus *Criconemoides* Taylor, 1936, which could not be identified by using the key presented by RASKI (1958) and is described herein as a new species. Subsequently, four living female specimens of this same nematode were recovered from dormant rose plants purchased by a commercial rose grower from a Pacific Coast supplier.

In addition to the characters used previously to determine species of *Criconemoides*, RASKI (1952) pointed out the importance of the presence or the absence of sub-lateral lobes in the lip region and also advised use of the cuticular pattern on larval forms to differentiate species. Distinctive larval cuticular patterns have been observed by MENZEL (1917) in *C. heideri* (Stefanski, 1916) Taylor, 1936; by TAYLOR (1936) in *C. mutabile* Taylor, 1936; by CHITWOOD (1949) in *C. simile* (Cobb, 1918) Chitwood 1949; and by RASKI (1952, 1958) in *C. xenoplax* Raski, 1952, and in *C. ornatum* Raski, 1958.

The description that follows was developed from observations of living and preserved specimens. Drawings were made with the aid of camera lucida and from photomicrographs. Measurements, given as a mean ± standard error and range, were obtained from specimens fixed in FAA and then processed by the rapid method and mounted in glycerine (GOODEY, 1957).

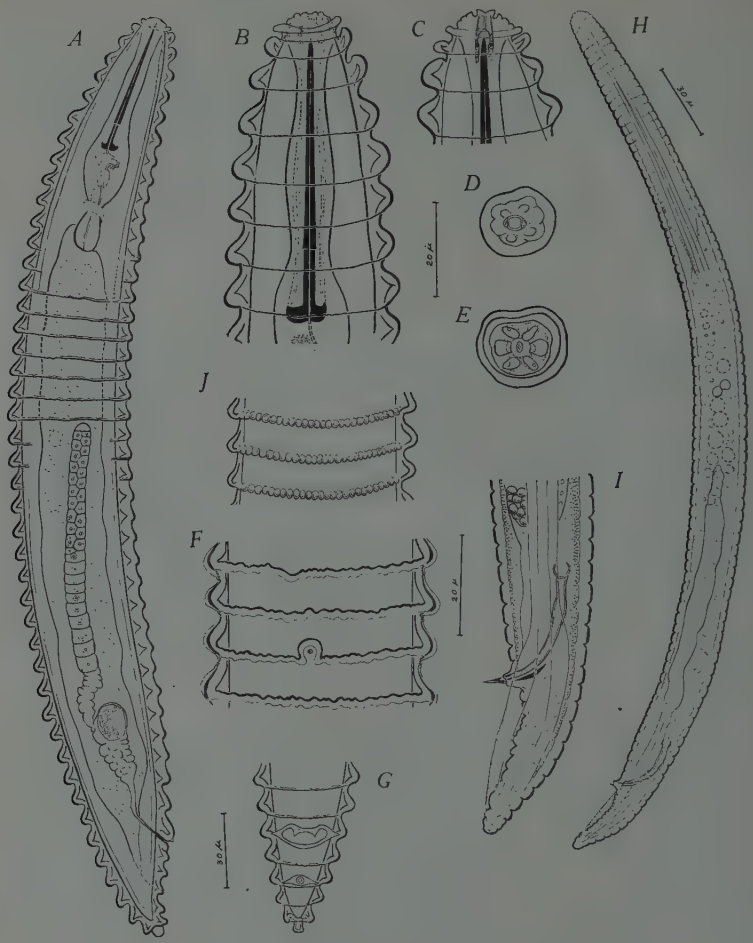

Fig. 1. *Criconemoides axeste* n. sp.

A. Female, lateral view.
B. Lateral view of anterior end, female.
C. Dorso-ventral view of anterior end, female.
D. Face view, female.
E. Head sclerotization, female.
F. Cuticle showing characteristic rough edge on posterior part of annules, ventral view, at region of excretory pore, female.
G. Female tail, ventral view.
H. Male.
I. Tail, male.
J. Larval cuticle showing beaded appearance of posterior part of annules.

Criconemoides axeste [1]) n. sp.

Female (n = 140) Length 0.407 ± .053 mm (0.320-0.525 mm); breadth 0.042 ± .005 mm (0.030-0.053 mm); a = 9.5 (7.7-11.7); b = 3.9 (3.8-4.2); c = 27.3 (24.4-30.6); V = 92% (90-96%); no. of body annules = 45 ± 3 (42-54); spear length = 54 ± 2 μ (51-60).

Male (n = 25) Length 0.350 ± .025 mm (0.298-0.394 mm); breadth 0.021 ± .003 mm (0.012-0.025 mm); a = 16.7 (12.9-27.1); b = ?; c = 13.2 (10.4-15.1); T [2]) = 42.3-53.9%.

Holotype — female — L = 0.425 mm; a = 10.9; b = 4.5; c = ?; V = 92.6%; stylet length 53 μ.

Allotype — male — L = 0.344 mm; a = 16.1; b = ?; c = 13.1; T = 43.6%

Female: (Fig. 1 A-G and Fig. 2A). The body is cylindrical, tapering gradually at anterior end and terminating in a rounded, blunt lip region. Posteriorly it tapers abruptly, ending in a conoid tail with a distinct knob. The amphid apertures are conspicuous in the dorso-ventral aspect, appearing as slits on lateral margins of labial disc. Four small sublateral lobes are present. These are obscure in lateral view but more discernible in face view and are equidistant around labial disc. The body annules are retrorse except for the anterior three. The first of these is bluntly rounded, smaller and narrower than the succeeding annule and usually projects forward on the ventral side. The second annule points anteriorly on the ventral side and either outwardly or less definitely forward on the dorsal side. The ventral side of the third annule points anteriorly while the dorsal side is retrorse. Annules are about 8 μ wide at the anterior end and 11 μ wide at the middle of the body. In a few specimens occasional anastomoses of annules occur. The posterior part of each annule exhibits a pronounced rough edge which apparently is derived from the oval cuticular markings on the larva. The spear is short and strong and extends through 7-9 annules. The excretory pore occurs on 12-15th annule from anterior end. No lateral fields are present. The vulva is on 4th or 5th annule from terminus and has an anterior flap which is prominent in lateral view. The flap presents a bilobed appearance in ventral aspect. From the vulva the vagina is directed obliquely forward and extends about halfway across the body. The uterus has a prominent, sperm-filled, spherical spermatheca that

1) From the Greek ἄξεστος rough-edged, in reference to the posterior edge of the annules of the female.

2) $T = \frac{\text{Distance from tail tip to anterior-most extension of testis}}{\text{Total body length}} \times 100$

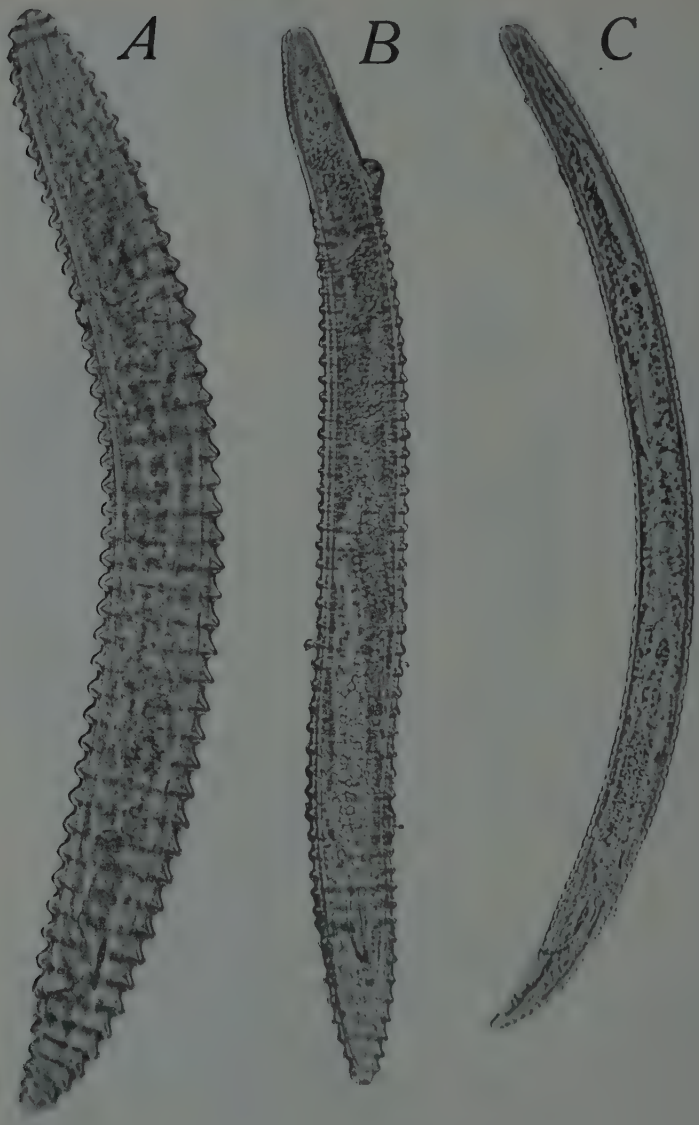

Fig. 2. *Criconemoides axeste* n. sp. x 400.

A. Adult female. B. Male in final molt. C. Adult male.

is especially evident in specimens mounted in glycerine. The ovary is variable in length; in mature females the blind end may extend to the median bulb. The anus is located on 2nd or 3rd annule from terminus; it is obscure in lateral view, but prominent in ventral aspect.

Male: (Fig. 1 H, I and Fig. 2 B, C). Males are rare, constituting about 1.5% of the population in two collections. They have little resemblance to the females, but could be identified positively since some were found in ecdysis (Fig. 2B). The lip region is low and rounded, and not offset. Flat annulations extend to lip region. They are 1.6 μ wide near the anterior end and 4.8 μ wide in middle of the body. A spear is absent and the esophagus indistinct. The lateral field is about one-third as wide as body, and is marked by 4 longitudinal striae, beginning as 2 just below the lip region. Faint cross striae are present irregularly in the anterior end of body. The hemizonid is about 3 μ long, located 2 annules anterior to the excretory pore. The spicules are 27-35 μ long and curved. The gubernaculum is small and trough-like. A spicule sheath protrudes conspicuously, bearing a short spine-like process on the posterior edge. This spine is obscured when spicules are extended. The small bursa begins slightly anterior to the cloacal opening as an extension of each sub-lateral line, and extends almost to the terminus.

Larva: The number of annules on the larvae fall within the same range as the number on the females. The posterior edges of the annules have an oval beaded appearance. (Fig. 1 J).

Type specimens — Holotype — female, collected by C. E. Williamson, January 10, 1957. Type slide number 15, deposited in the collection of the Nematology Section, Beltsville, Maryland. Allotype — male from collection made August 5, 1958. Type slide number 16, deposited in the collection of the Nematology Section, Beltsville, Maryland.

Paratypes — same data as holotype. Deposited in the collection of the Nematology Section, about 200 specimens in glycerine.

Type habitat — In soil about roots of roses in New York State greenhouses.

Type locality — In rose greenhouses in Bayport, Long Island, New York.

Distribution — This species was collected from several rose greenhouses on Long Island and in upstate New York.

Diagnosis — *Criconemoides axeste* n. sp. has all the generic characters of *Criconemoides* as outlined by TAYLOR (1936).

The female is distinguished from the described species of *Criconemoides* by the smaller number of annules (45 as compared with about

60 in *C. informe* (Micoletzky, 1922) Taylor, 1936), by the forward projecting 2nd and 3rd annules on the ventral side and by the rough posterior edge of the annules. Larvae are distinguished by the oval beaded cuticular pattern on posterior part of annules.

ZUSAMMENFASSUNG

Circonemoides axeste *n. sp. an Rosen in kommerziellen Glashäusern des Staates New York.*

Eine grosse Anzahl von *Criconemoides axeste* n. sp. wurde in Rosenwurzeln und in der Erde kommerzieller Glashäuser gefunden. Das Weibchen unterscheidet sich durch eine kleinere Anzahl von Ringeln (45 im Vergleich zu ungefähr 60 bei *C. informe* (Micoletzky, 1922) Taylor, 1936) sowie durch die vorstehenden 2ten und 3ten Ringel an der Ventralseite und durch ihren rauhen posterioren Rand. Larven zeichnen sich durch ein ovales, perlschnurförmiges kutikulares Muster am posterioren Teil der Ringel aus. Es wurde gefunden, dass die Männchen 1,5% der Nematodenbevölkerung ausmachen.

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DATA TO BE CONSIDERED, OBSERVED AND, WHERE POSSIBLE, REPORTED UPON WHEN PRESENTING DESCRIPTIONS OF NEW SPECIES

BY

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As the study of nematodes proceeds new species are continually being described. Many descriptions published today lack certain details which, although when described may not appear important, nevertheless give a fuller and more rounded picture of the species and may later become of importance. It seemed that a useful purpose would be served by compiling a list of all the points that need to be observed, considered and reported upon when a new species is described in a paper. Not all points in the following list will apply to all nematodes, and it may well be found that the list is not exhaustive, but it gives a useful basis on which a fairly complete description can be made.

No attempt is made to suggest any set form in which the actual data should be presented for that would destroy the freedom of expression and individuality of each author. However, it is suggested that, as far as possible, conciseness and accuracy of statement should go hand-in-hand. All statements should be unambiguous. By this I mean the statement which says: "larger than so-and-so" is ambiguous, but "1.7-2.2 times as long as so-and-so" admits of no doubt while allowing for variation.

In the actual form of words used a telegraphic style is helpful, occupies less space, uses less print, and has the additional merit of requiring the author to consider each statement very carefully before making it. This tends to ensure that the statement really says what the author intends it to mean and also obviates that verbosity which is frequently a sign and cause of ambiguity.

FIGURES

The description of a species can be kept to a minimum by the use of drawings. Figures can often show details of structure that are impossible to describe in words. Clear, unambiguous figures are absolutely

essential for the complete description of a new species. The minimum necessary are: —

1. A full-length figure of each sex.
2. A lateral view of the head end for the stoma or spear and its relationships to the lips and oesophagus.
3. An end-on-view of the head (so-called *en face*).
4. Transverse section through body at level of basal plate, and at other levels of head if necessary.
5. Transverse section through mid-body region for incisures.
6. Vulva in ventral view.
7. Spicules and associated structures in lateral view.
8. The same in ventral view.
9. Any feature of special interest or structure should be figured from more than one aspect. Any important variations should be adequately figured.

All the figures should be of generous magnification, with no more than the minimum of detail to make them clear and pleasing to those who will read the paper.

Magnifications are best cited by scales in the figures, with clear indication of the drawings to which each scale refers.

As examples of the above regarding figures the following come up to the standards suggested [1]):

Thorne, G. (1949). On the classification of the *Tylenchida,* new order (Nematoda, Phasmidia). *Proc. helminth. Soc. Wash.* **16** (2), 37-73. [See figs. 3 & 8.]

Sher, S. A. & Allen, M. W. (1953). Revision of the genus *Pratylenchus* (Nematoda; Tylenchidae). *Univ. Calif. Publ. Zool.* **57** (6), 441-470. [See plate 66.]

SOURCE AND TREATMENT OF MATERIAL

How and from where obtained; a rough idea of numbers found.

How long from full activity to examination and also to fixation.

Condition of specimens examined and particularly of those measured, because of shrinkage (up to 20 %) which may occur in some fixatives and during processing.

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1) Goodey, J. B. (1957). *Hoplolaimus proporicus* n. sp. (Hoplolaiminae: Tylenchida). *Nematologica* 2 (2), 108-113 would serve equally as an example. (*Editor's Note*).

Method of fixation after killing by just sufficient heat.
Method of processing. State of those specimens used for drawing.

MEASUREMENTS OF SPECIMENS

n ♀ ♀ L = mean length in mm to 2 places decimals (s = ± standard error to 3 places decimals) (range =)
[it is doubtful whether greater accuracy is possible]
a = length ÷ greatest width (range)
b = length ÷ distance from anterior end to junction of oesophagus and intestine (range)
[n.b. where the glandular terminal bulb of the oesophagus overlaps the intestine particular care has to be taken over the data for *b*. Quite frequently the distance measured is to the end of the overlapping glandular lobe: if this is done it should be very clearly stated, e.g. species of *Aphelenchoides, Rotylenchus.*]
c = length ÷ tail length (range)
[n.b. the tail is strictly to be measured from anus to the terminal end of the body.]

	anterior gonad as % body length (range)		posterior gonad as % body length (range)
V =		Vulva % position (range)	

n ♂ ♂ L = similar data to above
a, b & c similar to above. T = gonad as % body length, (range).
Holotype ♀ — actual data of specimen — L, a, b, c & V.
Allotype ♂ — actual data of specimen — L, a, b, c & T.

As an example, the above data are given as set out for *Paraphelenchus myceliophthorus.*

Paraphelenchus myceliophthorus Goodey, 1958

50 ♀♀ L = 0.70 mm (s = ± 0.058 mm) (range 0.58-0.82 mm)
a = 22-34; b = 4.1-6.6; c = 13-24; V = $^{26\text{-}55}$ 71-78 $^{9\text{-}15}$
50 ♂ ♂ L = 0.69 mm (s = ± 0.060 mm) (range 0.56-0.82 mm)
a = 23-32; b = 3.9-5.9; c = 14-30; T = 46-77
Holotype ♀ L = 0.79 mm; a = 26; b = 4.8; c = 18; V = ^{36} 75 $^{10.6}$
Allotype ♂ L = 0.79 mm; a = 24; b = 5.6; c = 22; T = 64

Sexual dimorphism. Ratio of ♀ to ♂

DETAILS TO BE OBSERVED

In observing the following features the best microscope possible should be used. Observation by ordinary transmitted light should be supplemented by observation with incident illumination, which shows many surface details more clearly.

Outside of body

Cuticle — thickness; striations, their type and width;
— lateral fields — general width as fraction of body width; number; course of variation in number from head to tail; pattern on ♂ tail; deirids;
— phasmids;
— pores on body, particularly on tail.

Subcuticle — thickness; striations.

Parts of body

Head — lateral view — offset; general shape and symmetry; how many annules; head cap; papillae or setae; amphids; head frame-work; spear guide.
— end-on view — general shape and symmetry; variation in size &/or shape between lips; papillae or setae; any sexual differences; amphids, size and position; head framework; spear guide.

Mouth stoma — lateral view — shape and dimensions;
Rhabdions-
Cheilo- } Shape and state of Cheilorhabdions.
Pro- } Shape, dimensions and relationships between different Rhabdions.
Meso- } Modifications to Metarhabdial segments as teeth, denticles, plates, etc.
Meta- }
Telo- } Modifications to other parts.
— end-on view — shape and dimensions, etc.

Mouth spear — what type (stomatostyle, odontostyle); fore part; hind part; base; shape of knobs; general appearance and dimensions.

Excretory pore — position; type of excretory system in as much detail as possible.

Ocelli

Hemizonid

Nerve ring — position relative to oesophagus and excretory pore.
Oesophagus — relationship of orifice of dorsal oesophageal gland to spear base;
— general shape; any associated muscular sheath; disposition of gland openings; position and number of gland nuclei;
— anterior part — shape; length, lumen; associated thickening;
— median bulb — shape and dimensions; valve shape and size; junction of dorsal oesophageal gland duct; junction of sub-ventral glands ducts;
— isthmus — length and shape;
— posterior bulb — shape and size; relationship to intestine; position and number of gland nuclei; valvular apparatus in considerable detail.
Oesophago-intestinal junction — minute valve present;
— oesophago-intestinal cells (so-called cardia); their number, shape, size and disposition.
Intestine — opacity; size of granular contents of cells.
Pre-rectum - length and shape from lateral and ventral views.
Rectum — length and shape from lateral and ventral views.
Anus — position and shape; post-anal diverticulum.

Female — state of gonad at maturity, (and when spermatized, if bisexual).
— vulva — position and orientation; shape (ventral view); association flaps of cuticle.
— vagina — disposition; thickness of walls.
— gonads — number and position; reflexed (how many times?); post-vulval sac.
— ovary; cells in how many rows.
— oviduct; structure.
— spermatheca &/ or spermogonium; shape and size.
— uterus; shape, size and thickness of walls.
— eggs; size and shape; number in uterus at a time; state at deposition.
— sperms in ♀; where.
— any other observations.

— tail — shape; length; phasmids (position and size); pores; glands; tip mucrones.

Male — any precocity.
— gonads — number and general disposition; any reflexion.
— testis; cells in how many rows; length and shape.
— vas deferens; length and shape.
— ratio of testis to vas deferens.
— sperms; shape and size; number.
— spicules; shape and size in very full detail.
— gubernaculum; shape and size in full detail.
— 'telamon' or other associated structures.
— associated muscular sheath, with whole or parts.
— ejaculatory glands.
— tail — shape; length; bursa in fullest detail; phasmids; pores; papillae in full detail for disposition; glands; spinneret.

Larvae — any differences from adults.

Differential diagnosis — concise account of how species differs from others in genus.

Type slides — full data of Holotype, Allotype and any other types; where deposited; catalogue numbers.

Type host —

Type habitat or locality as accurately as possible.

Any further comments.

New higher categories, including new genera — concise diagnosis and a differential diagnosis; reasons for setting up new category.

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BASIRIA GRAMINOPHILA N.G., N.SP., (NEMATODA: TYLENCHINAE) FOUND ASSOCIATED WITH GRASS ROOTS IN ALIGARH, INDIA

BY

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Various turf soil samples from pastures and lawns of Aligarh, India yielded considerable numbers of nematodes, in various stages of development, which could not be assigned to any known genus of the family Tylenchidae. Therefore a new genus, the description of which follows hereunder, is proposed for their reception.

Basiria [1], n.g.

Diagnosis. Tylenchinae: Body with conspicuous transverse striae which are interrupted by lateral fields bearing four incisures. Lip region elevated, set off by a slight narrowing of the neck contour. Labial frame-work not sclerotized. Amphid apertures large, conspicuous, slit-like, situated posteriorly to base of lateral lips. Deirids and phasmids easily seen. Spear slender, about 12 μ long, divisible into two unequel parts, an anterior tapering tip and a posterior cylindrical shaft bearing three symmetrical, rounded basal knobs. Orifice of the dorsal oesophageal gland, as in *Helicotylenchus*, situated more than half the spear-length behind the base of the spear. This is a unique feature among Tylenchinae. Distance from anterior end of body to centre of median oesophageal bulb greater than that from latter to base of oesophagus. Corpus ovate, with distinct valvular apparatus. Basal oesophageal bulb pyriform, set off from intestine. Cardia well developed. Vulva situated in posterior region of body. Ovary single, prodelphic, outstretched; oöcytes arranged in single file. Testis single, outstretched; bursa short, adanal; spicules paired, tylenchoid; gubernaculum simple, trough-shaped. Tails of both sexes elongate, filiform.

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[1]) Named after Dr. M. A. Basir under whose guidance this work was done.

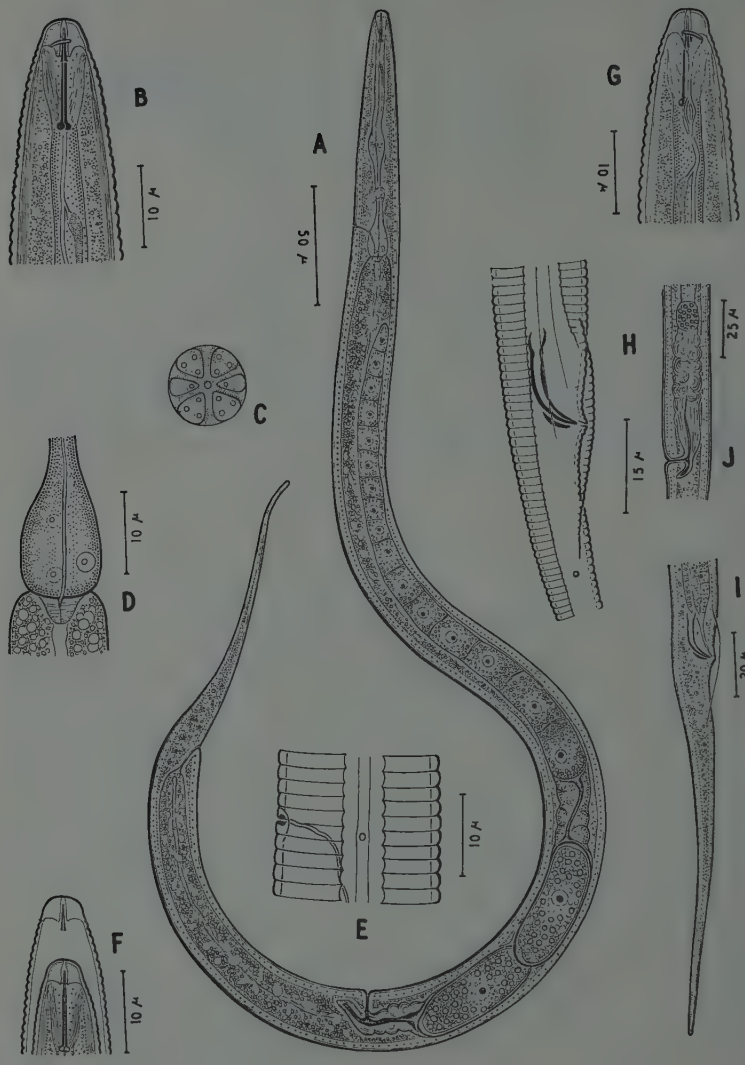

Fig. 1. *Basiria graminophila*: A — Female; B — Anterior end of female; C — *En face* view of female; D — Basal oesophageal bulb and cardia; E — Deirid region of female; F — Anterior end of moulting larva; G — Anterior end of male; H — Cuticular pattern of male tail; I — Tail end of male; J — Uterus with spermatheca.

Type species. *Basiria graminophila,* n.g. n.sp.

Basiria graminophila, n.g. n.sp.

(Figure 1, A-J)

35 ♀♀: L = 0.51-0.82 mm; a = 30-38; b = 5.4-7.7; c = 5.5-6.8; V = 61-67 %; spear = 11-12 μ; orifice of dorsal oesophageal gland 9-12 μ from spear base.

20 ♂♂: L = 0.51-0.70 mm; a = 35-40; b = 5.4-7.4; c = 4.5-6.2; T = 31-48%; spear = 10-12 μ; spicules = 16-19 μ; gubernaculum = 4-5.5 μ.

Female (Holotype): L = 0.81 mm; a = 30.3; b = 7.7; c = 6.7; V = $^{45}64.3^{1.6}$; spear 12 μ; orifice of dorsal oesophageal gland 9 μ from spear base.

Body long, slender and gradually tapering towards the extremities. When relaxed by gentle heat, the eelworms become slightly ventrally curved but unless properly relaxed the tail becomes irregularly bent. Body cuticle with distinct transverse striae 1.6 μ and 1.2 μ apart on mid-body and tail region respectively. No striae on the head although a slight indication of four in certain paratypes. Body striae are completely interrupted by lateral fields which have four distinct incisures; outer incisures distinctly crenate. Lateral fields two-sevenths of body-width. They originate slightly posterior to spear base as two incisures which gradually widen out to have two more between them over the median oesophageal bulb. Posteriorly the four incisures run almost parallel till they disappear near middle of tail. Lateral fields bear distinct deirids at level of hemizonid and phasmids on tail about two anal-body-diameters behind anus.

Lip region is elevated, cupolate, bluntly rounded anteriorly and set off by slight narrowing of the neck contour. It is 6.5 μ broad at its base and 3.3 μ high and is supported by a hexaradiate, non-sclerotized framework. In an *en face* view (Fig. 1, C) six completely amalgamated lips bordering the small, centrally located oral opening can be seen. Lateral lips are smaller than sub-median lips which are heart-shaped. Labial papillae are located as shown. Large, slit-like amphid apertures are located behind base of lateral lips.

Buccal spear 12 μ long comprised of two unequal parts, an anterior, shorter, tapering tip one-third of length and a posterior, longer, cylindrical shaft which bears three symmetrical, rounded basal knobs, which measure 1.6 μ across. Inner margins of labial frame-work form a short

guiding tube for spear. The precorpus is cylindrical, 4 μ in diameter, with outlet of dorsal oesophageal gland 9 μ behind spear base. Duct of gland is distinctly cuticularized near its opening into oesophageal lumen. Median oesophageal bulb ovate, 10 μ long by 6.5 μ wide: muscular and contains a distinct valvular apparatus just behind which open sub-ventral oesophageal glands. Distance from anterior end of body to middle of corpus is in ratio of 5 : 4 to latter to base of oesophagus. Isthmus cylindrical and slightly narrower than precorpus. It expands to form a pyriform basal oesophageal bulb, completely set off from intestine and containing three oesophageal gland nuclei of which the dorsal is largest and most distinct. Oesophageal lumen in basal oesophageal bulb is well defined. Nerve ring crosses middle of isthmus. Excretory duct quite distinct. Excretory pore strongly sclerotized, located ventrally at level of beginning of basal oesophageal bulb, 16 μ behind nerve ring. In lateral view hemizonid appears as a transparent area extending three body annules at level of deirids (Fig. 1, E). Cardia is large and conoid. Intestine has a small, well defined lumen throughout; intestinal cells are packed with rounded, refractive granules of varying sizes causing live eelworms to appear opaque and dark.

Vulva a depressed transverse slit, occupying two-fifths of body diameter. Vagina short, about one-third body diameter long. There is a short, posterior uterine branch about half a vulval-body-width. Uterus highly muscular, contains two well-developed but unsegmented eggs which measure 56 μ by 20 μ. Ovary outstretched anteriorly, its oöcytes in single file. A spherical spermatheca, containing sperms, is present at distal end of uterus and beyond it a short oviduct connects it to ovary. (Fig. 1, J). Vulva-anus distance greater than tail length.

Rectum is 13 μ long and opens through a conspicuous anus. Tail filiform, tapering regularly to a smoothly rounded terminus, and is nine anal-body-diameters long: it is distinctly striated.

Male (Allotype). Length = 0.69 mm; a = 36.3; b = 6; c = 5.9; T = 40 %.

Male essentially similar to female but body striations about 1.3 μ apart; lateral field expands on tail as in fig. H; head not striated; spear 11 μ long; orifice of dorsal oesophageal gland 9 μ from spear base. Amphidial duct forms a globular pouch packed with hair-like sensillae, at level of spear base (Fig. 1, G.).

Testis single and outstretched. Behind cap-cell are two spermatogonia in single file then in double rows till amoeboid spermatozoa are formed. A small, adanal bursa originates slightly anterior to head of spicules

and terminates near phasmids; its edges distinctly crenate. Copulatory spicules paired, similar, ventrally arcuate and distinctly cephalated; 18.5 μ long and resting on a simple, trough-shaped gubernaculum, 5 μ long.

Rectum short, opening through a distinct cloaca situated on a prominent protuberance of the body.

Holotype. Female specimen collected on 15th December, 1958; slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

Allotype. Male specimen collected around roots of grass; other data same as for holotype.

Paratypes. Hundreds of males and females collected around grass and citrus roots; other data same as for holotype.

Type habitat. Collected from soil around roots of grass, *Cynodon dactylon* Pers.

Type locality. Aligarh (U.P.), India.

Hosts and geographical distribution. Specimens of this species have been collected from soil around pasture and lawn grass roots in various localities in Aligarh and Mathura districts of Uttar Pradesh (North India); also around citrus roots (*Citrus limon* (L.) Burm. and *C. aurantium* L.) in Aligarh and Banda districts of the same state. It is presumed that the species is widely distributed in North India.

Bionomics. In water the eelworms show a slow but graceful movement of their bodies resembling those of plant-parasitic species of the genera *Tylenchorhynchus, Pratylenchus, Ditylenchus* etc. The refractive, spherical dense food granules of the intestine also resemble those found in the various plant-parasitic species. No specimens have been found within the plant roots and it is, therefore, presumed that the worms live permanently in soil and feed on roots from outside, or on associated fungal hyphae of the rhizosphere.

DISCUSSION

Basiria n.g. is closely allied to *Psilenchus* de Man, 1921 of the Tylenchinae, from which its essential difference is the position of the orifice of the dorsal oesophageal gland at three-quarter to one spear length from the spear base. *Psilenchus* itself contains only a few species and shows considerable morphological diversity, e.g. lip region with or without striae, spear with or without plain or asymmetrical knobs, single or paired ovaries etc. The finding of further species may well lead to a division of the genus.

Basiria is the only genus of the Tylenchinae in which the dorsal oesophageal gland opens some distance behind the spear base. In the Hoplolaiminae, *Helicotylenchus* Steiner, 1945 is distinguished in a similar way and in the Nacobbinae *Rotylenchus* Linford & Oliveira 1940 has the orifice halfway between spear base and median bulb.

The slit-like amphid apertures are found in *Psilenchus* and in *Tylenchus* Bastian, 1865 as well as in *Basiria*. The three genera have considerable affinities and may well have been derived from a common ancestral stock.

Acknowledgement. The author is thankful to Dr. J. B. Goodey for going through the paper and making valuable suggestions which have been gratefully accepted.

ZUSAMMENFASSUNG

Basiria graminophila n.g. n.sp., die in Aligarh, Indien, an Graswurzeln — *Cynodon dactylon* Pers. — gefunden wurde, wird beschrieben und abgebildet. *Basiria* n.g. gehört zur Unterfamilie der Tylenchinae und gleicht am meistem der Gattung *Psilenchus* De Man, 1921; von dieser unterscheidet sie sich durch die Lage der Öffnung der Ösophagusdrüse, welche auf 3/4 bis eine Stachellänge hinter der Basis des Stachels liegt; diese Eigenschaft ist einzigartig unter den Tylenchinae.

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NEUBENENNUNGEN EINIGER HOMONYMEN NEMATODEN-GATTUNGEN

VON

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Nach dem Durchblättern des nomenklaturischen Gattungsregisters von SCHULZE, KÜKENTHAL & HEIDER (Nomenclator animalium generum et subgenerum. Berlin, 1-5, 1926-1940, pp. 3716) und der betreffenden Bände von Zoological Record konnte ich feststellen, dass einige Gattungen der freilebenden Nematoden homonym sind, d.h. ihre Namen bereits für andere Tiergattungen in Anspruch genommen wurden. Diese Nematoden-Gattungen sind *Trilobus* Bastian, 1865, *Greenia* Hoeppli & Chu, 1932 und *Trachypleura* Thorne, 1939. Im Sinne der Prioritätsregel versehe ich diese Gattungen mit neuen generischen Namen.

Die Gattung *Trilobus* wurde von BRÜNNICH bereits im Jahre 1781 für fossile Trilobiten (Crustacea) aufgestellt (Nye Saml. Kongl. Danske Vid. Selsk. Skr. 1. p. 388). Der von BASTIAN benutzte Name *"Trilobus"* ist also ein Homonym. Ich schlage daher für die hierher gehörigen Arten den neuen Gattungsnamen *Tobrilus* [1]) vor.

Der Name *Greenia* war vor HOEPPLI & CHU bereits von zwei Verfassern in Anspruch genommen. Im Jahre 1896 versah KIRBY eine Gattung der Phasmodeen (Orthoptera) mit diesem Namen (Trans. Linn. Soc. London, Zool. 6, p. 456), während 5 Jahre später OUDEMANS eine Gattung der Gamasiden (Acarina) mit demselben Namen bezeichnet hat (Tijdschr. Nederl. Dierk. Ver. 7, p. 52 & 60). Da der KIRBY-sche Name die Priorität geniesst, versehe ich die Gattung von HOEPPLI & CHU mit dem neuen Namen *Greenenema*.

Der Gattungsname *Trachypleura* wurde das erste Mal von JAEKEL gebraucht, der im Jahre 1900 eine fossile Chitonide-Gattung (Mollusca) mit diesem Namen bezeichnete (Zeitschr. Deutsch. Geol. Ges. 52. p. 9). Da also der von THORNE im Jahre 1939 einer Nematoden-Gattung gegebene Name *Trachypleura* als Homonym betrachtet werden

1) Ist ein Anagramm von *Trilobus*.

muss, benenne ich die hierher gehörigen Arten mit dem neuen Gattungsnamen *Trachypleurosum*.

Die neuen Namen der betreffenden Gattungen und Arten sind also wie folgt:

Tobrilus n. nom.

(Syn.: *Trilobus* Bastian, 1865 nec *Trilobus* Brünnich, 1781)

42 Arten [1]):

Tobrilus aberrans (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis* var. *aberrans* W. Schneider, 1925.

Tobrilus aequiseta (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis* var. *aequiseta* W. Schneider, 1925.

Tobrilus allophysis (Steiner, 1919) n. comb. — Syn.: *Trilobus gracilis* var. *allophysis* Steiner, 1919.

Tobrilus allophysoides (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis allophysis* var. *allophysoides* W. Schneider, 1925.

Tobrilus altherri n. nom. — Syn.: *Trilobus allophysoides* Altherr, 1953 nec *T. gracilis allophysis* var. *allophysoides* W. Schneider, 1925.

Tobrilus armatus (Allgén, 1925) n. comb. — Syn.: *Trilobus armatus* Allgén, 1925.

Tobrilus birói (Daday, 1899) n. comb. — Syn.: *Trilobus birói* Daday, 1899.

Tobrilus brevisetosus (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis* var. *brevisetosus* W. Schneider, 1925.

Tobrilus conjungens (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis* var. *conjungens* W. Schneider, 1925.

Tobrilus consimilis (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis medius* var. *consimilis* W. Schneider, 1925.

Tobrilus diversipapillatus (Daday, 1905) n. comb. — Syn.: *Trilobus diversipapillatus* Daday, 1905.

Tobrilus dryophilus (Rahm, 1925) n. comb. — Syn.: *Trilobus dryophilus* Rahm, 1925.

Tobrilus gracilis (Bastian, 1865) n. comb. — Syn.: *Trilobus gracilis* Bastian, 1865.

Tobrilus graciloides (Daday, 1908) n. comb. — Syn.: *Trilobus graciloides* Daday, 1908.

Tobrilus grandipapillatus (Brakenhoff, 1914) n. comb. — Syn.: *Trilobus grandipapillatus* Brakenhoff, 1914.

Tobrilus helveticus (Hofmänner, 1915) n. comb. — Syn.: *Trilobus helveticus* Hofmänner, 1915.

Tobrilus homophysalides (Steiner, 1919) n. comb. — Syn.: *Trilobus gracilis* var. *homophysalides* Steiner, 1919.

Tobrilus höhnelensis (Allgén, 1952) n. comb. — Syn.: *Trilobus höhnelensis* Allgén, 1952.

1) Unter den hier angeführten 42 Arten gibt es wahrscheinlich auch solche, die Synonyme anderer *Tobrilus*-Arten sind. Da aber die diesbezüglichen Meinungen der verschiedenen Verfasser voneinander sehr abweichen, zähle ich alle beschriebenen Arten bzw. Formen einstweilen als gültige Arten auf, mit der Bemerkung, dass die Arten der Gattung *Tobrilus* einer starken Revision bedürfen.

Tobrilus husmanni (Altherr, 1958) n. comb. — Syn.: *Trilobus husmanni* Altherr, 1958.

Tobrilus imberbis (Andrássy, 1953) n. comb. — Syn.: *Trilobus imberbis* Andrássy, 1953.

Tobrilus leptosoma (de Man, 1880) n. comb. — Syn.: *Trilobus leptosoma* de Man, 1880.

Tobrilus lomnickii (Grochmalicki, 1911) n. comb. — Syn.: *Trilobus lomnickii* Grochmalicki, 1911.

Tobrilus longicauda (Linstow, 1876) n. comb. — Syn.: *Trilobus longicauda* Linstow, 1876.

Tobrilus longicaudatus (W. Schneider, 1923) n. comb. — Syn.: *Trilobus gracilis* f. *typicus* sf. *longicaudatus* W. Schneider, 1923.

Tobrilus longus (Leidy, 1851) n. comb. — Syn.: *Anguillula longa* Leidy, 1851; *Trilobus longus* (Leidy, 1851) Bastian, 1865.

Tobrilus malayanus (W. Schneider, 1937) n. comb. — Syn.: *Trilobus malayanus* W. Schneider, 1937.

Tobrilus medius (G. Schneider, 1916) n. comb. — Syn.: *Trilobus medius* G. Schneider, 1916.

Tobrilus murisieri (Altherr, 1938) n. comb. — Syn.: *Trilobus murisieri* Altherr, 1938.

Tobrilus octiespapillatus (Linstow, 1876) n. comb. — Syn.: *Trilobus octiespapillatus* Linstow, 1876.

Tobrilus paludicola (Micoletzky, 1925) n. comb. — Syn.: *Trilobus stefanskii* var. *paludicola* Micoletzky, 1925.

Tobrilus parvipapillatus (Kreis, 1932) n. comb. — Syn.: *Trilobus parvipapillatus* Kreis, 1932.

Tobrilus pellucidus (Bastian, 1865) n. comb. — Syn.: *Trilobus pellucidus* Bastian, 1865.

Tobrilus pseudallophysis (Micoletzky, 1925) n. comb. — Syn.: *Trilobus pseudallophysis* Micoletzky, 1925.

Tobrilus punctatus (Stefanski, 1916) n. comb. — Syn.: *Trilobus gracilis* var. *punctata* Stefanski, 1916.

Tobrilus scallensis (W. Schneider, 1925) n. comb. — Syn.: *Trilobus gracilis scallensis* W. Schneider, 1925.

Tobrilus stefanskii (Micoletzky, 1925) n. comb. — Syn.: *Trilobus stefanskii* Micoletzky, 1925.

Tobrilus steineri (Micoletzky, 1925) n. comb. — Syn.: *Trilobus steineri* Micoletzky, 1925.

Tobrilus telekiensis (Allgén, 1952) n. comb. — Syn.: *Trilobus telekiensis* Allgén, 1952.

Tobrilus tenuicaudatus (Daday, 1894) n. comb. — Syn.: *Trilobus tenuicaudatus* Daday, 1894.

Tobrilus wesenbergi (Micoletzky, 1925) n. comb. — Syn.: *Trilobus wesenbergi* Micoletzky, 1925.

Tobrilus zakopanensis (Stefanski, 1924) n. comb. — Syn.: *Trilobus gracilis* var. *zakopanensis* Stefanski, 1924.

Greenenema n. nom.

(Syn.: *Greenia* Hoeppli & Chu, 1932 nec *Greenia* Kirby, 1896 et nec *Greenia* Oudemans, 1901).

1 Art:

Greenenema orientale (Hoeppli & Chu, 1932) n. comb. — Syn.: *Greenia orientalis* Hoeppli & Chu, 1932.

Trachypleurosum n. nom.

(Syn.: *Trachypleura* Thorne, 1939 nec *Trachypleura* Jaekel, 1900).

2 Arten:

Trachypleurosum conforme (W. Schneider, 1935) n. comb. — Syn.: *Actinolaimus conformis* W. Schneider, 1935; *Trachypleura conformis* (W. Schneider, 1935) Thorne, 1939.

Trachypleurosum labyrinthostoma (Cobb, 1893) n. comb. — Syn.: *Dorylaimus labyrinthostoma* Cobb, 1893; *Actinolaimus labyrinthostoma* (Cobb, 1983) Cobb, 1913; *Trachypleura labyrinthostoma* (Cobb, 1893) Thorne, 1939.

SUMMARY

Three homonymic genera of free-living nematodes are renamed. The new generic names are: *Tobrilus* n. nom. for *Trilobus* Bastian, 1865 (not *Trilobus* Brünnich, 1781), *Greenenema* n. nom. for *Greenia* Hoeppli & Chu, 1932 (not *Greenia* Kirby, 1896, or *Greenia* Oudemans, 1901) and *Trachypleurosum* n. nom. for *Trachypleura* Thorne, 1939 (not *Trachypleura* Jaekel, 1900). All species of these renamed genera are also listed.

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A COMPARISON OF THE RATES OF EMERGENCE AND INVASION OF BEET EELWORM *HETERODERA SCHACHTII* SCHMIDT AND PEA ROOT EELWORM *HETERODERA GÖTTINGIANA* LIEBSCHER

BY

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The relationship between hydrostatic pressure deficiency or suction and the emergence of larvae of the beet eelworm from cysts and their subsequent migration through the soil has been discussed previously (Wallace 1954, 1955). Little is known, however, about the influence of suction on the invasion of host plant roots and no reference is known to the influence of soil physical factors on the emergence, mobility and invasion of the pea root eelworm. These two species of *Heterodera* differ in their responses to root diffusates from the host plant, beet eelworm has an increased emergence rate in the presence of diffusates relative to the emergence in water (Winslow 1954), whilst pea root eelworm exhibits little if any response to such stimulation *in vitro*. Under field conditions, however, both species respond to the presence of host roots by an increased emergence rate (Brown 1958, Jones & Moriarty 1956, Jones 1956).

In this paper, the emergence and rate of invasion of beet and pea root eelworms is compared in sand at known suctions in the presence of the roots of their respective hosts. Mention is also made of the influence of suction on diffusate production in cress [1]), a host of beet eelworm, and of the diffusion of root diffusate through a coarsely particulate medium. On the basis of these results a hypothesis is put forward to account for differences in behaviour between the two species and to suggest how these differences may influence larval and cyst numbers in the soil.

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1) *Lepidium sativum* L.

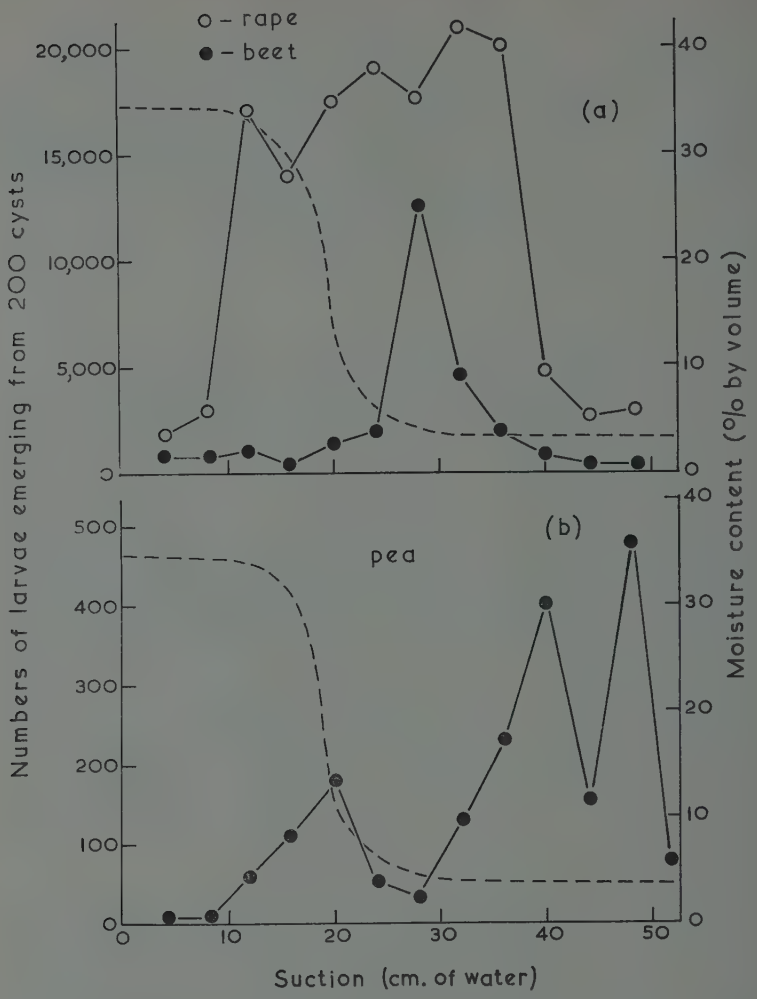

Fig. 1. The influence of suction on the emergence of larvae from cysts of (a) beet eelworm and (b) pea root eelworm in the presence of host plant roots. The dotted curve indicates the moisture characteristic.

RESULTS

Emergence and Invasion

Experiments were performed in sintered glass funnels 9 cm in diameter with sand of 250-500 μ particle size. The control of suction and

methods for the recovery and counting of larvae have already been described (WALLACE 1954). Numbers of larvae within roots were determined using the Waring Blendor technique (GOODEY 1957). The interpretation of soil moisture in terms of suction together with a description of the properties of the moisture characteristic has been described by WALLACE (1955, 1958).

To determine the influence of host plant roots on the emergence rate of larvae from cysts, 6 rape [1]) seeds were placed on either side of a row of 200 beet eelworm cysts at a ¼ inch depth. Twelve different suctions were used ranging from 4 to 48 cm of water, the depth of the sand in the funnel being one inch. After two weeks the numbers of larvae in the sand and in the roots at each suction were determined. A similar experiment was then performed using four beet seed clusters. The results are given in Fig. 1a. A further experiment was done with pea root eelworm under similar experimental conditions except that 4 pea seeds were used. These results are expressed in Fig. 1b. The results suggest that both species have an optimal suction range for emergence but because of the low rate of emergence for pea root eelworm the variability was high. In the presence of rape roots the emergence rate is at a maximum over the relatively broad suction range of 12-36 cm of water whilst with beet roots the range is more defined at 24-32 cm of water. This difference in the form of the two curves is probably attributable to a difference in root growth with suction between the two species. Both germination and root growth were poor in beet compared with the rape. With the pea root eelworm cysts, an increased rate of emergence occurred in the presence of the pea roots, the maximum number emerging in the absence of roots at any one suction being eighteen. Furthermore, there is an optimum suction range for emergence between 32-52 cm of water which is higher than that for beet eelworm. In terms of the percentage larvae emerging, pea-root eelworm with a maximum of 2% in two weeks is considerably less than beet eelworm with 50%.

The salient points from these experiments are:

(1) Both species have an optimum suction range for emergence which is higher for pea root eelworm than beet eelworm.

(2) In the presence of host roots beet eelworm has a considerably higher rate of emergence than pea root eelworm.

The data from the experiments just described yield information about

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1) *Brassica napus* L., var. *oleifera* D. C.

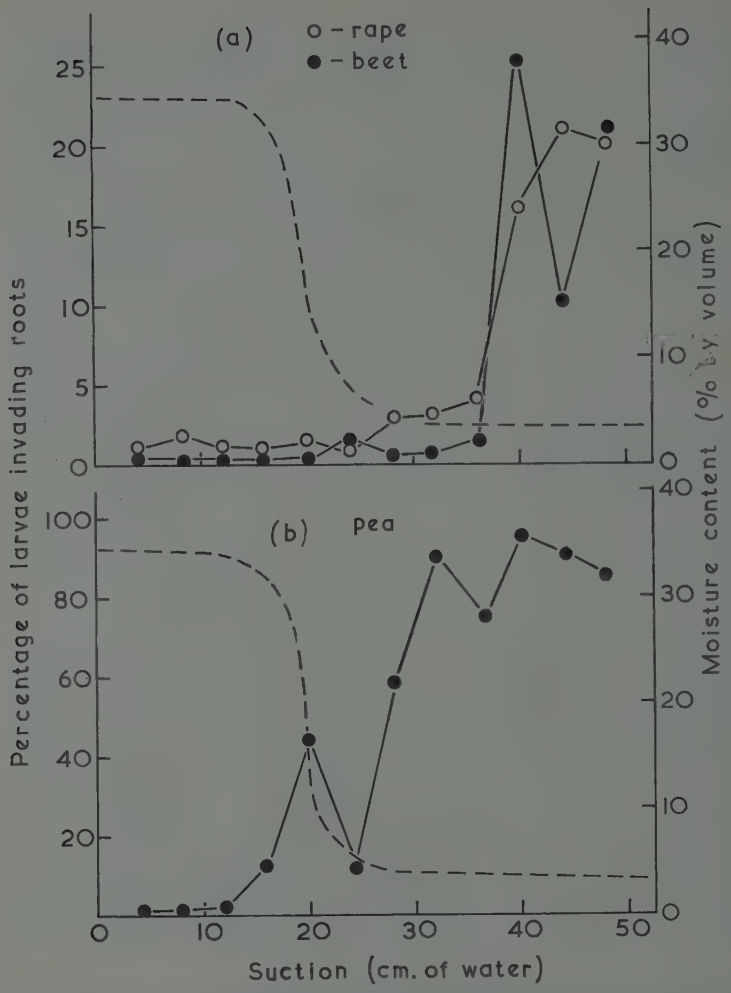

Fig. 2. The influence of suction on the invasion rate of larvae of (a) beet eelworm (b) pea root eelworm in the presence of host plant roots. The dotted curve indicates the moisture characteristic.

percentage invasion as well as total emergence rate. When the numbers of larvae recovered from roots are expressed as percentages of the total numbers of larvae emerging and then plotted against suction, the graphs shown in Fig. 2 are obtained. Under these conditions the rate of

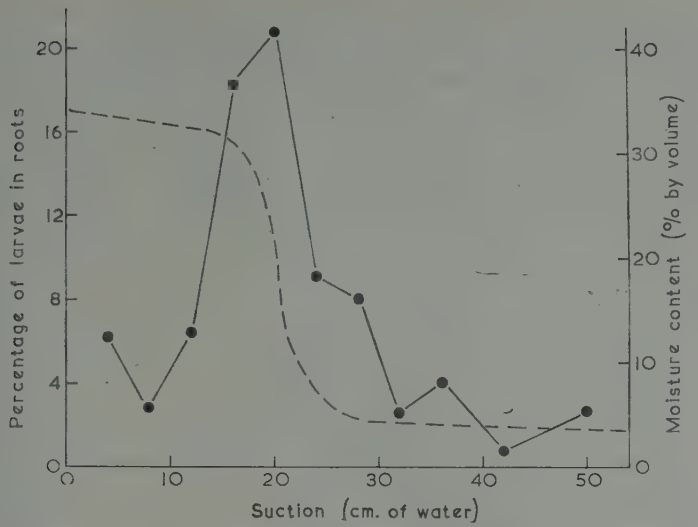

Fig. 3. The influence of suction on the invasion of cress roots by beet eelworm. The dotted curve indicates the moisture characteristic.

invasion of beet eelworm in the rape and beet never exceeds about 25% (Fig. 2a). This was confirmed in another experiment where larvae instead of cysts were introduced into the sand. The results from this experiment (Fig. 3) differ from the previous experiment in that there was an optimum suction for invasion at about 16-24 cm of water but once again invasion reached a maximum at about 20%. With pea-root eelworm the percentage invasion increased with suction to about 95% (Fig. 2b).

Thus, the results suggest that pea-root eelworm has a considerably higher invasion rate than beet eelworm.

Diffusate production and the diffusion of root diffusate

In a previous paragraph it has been mentioned that root growth may vary according to suction. This in turn may influence the production of diffusate and, to examine this possibility, cress seed was grown in sand in six sintered funnels at different suctions for one week. The whole of the water in each of the funnels was then removed and made up to give equal volumes. Eight batches of 100 cysts were placed in each of the six solutions and the rate of emergence of beet eelworm was determined over two weeks. The results (Fig. 4) indicate that

there is an optimum suction for diffusate production in cress roots. The optimal soil moisture conditions for root growth and diffusate production probably vary with species so it would be unwise to assume that other plants behave in a similar way to cress. However, the point is made that soil suction probably influences emergence by its effect on the plant (diffusate production) as well as by a direct physical process. Whether such a situation occurs in the relationship between pea root eelworm and its host plant has not been determined, the consistently

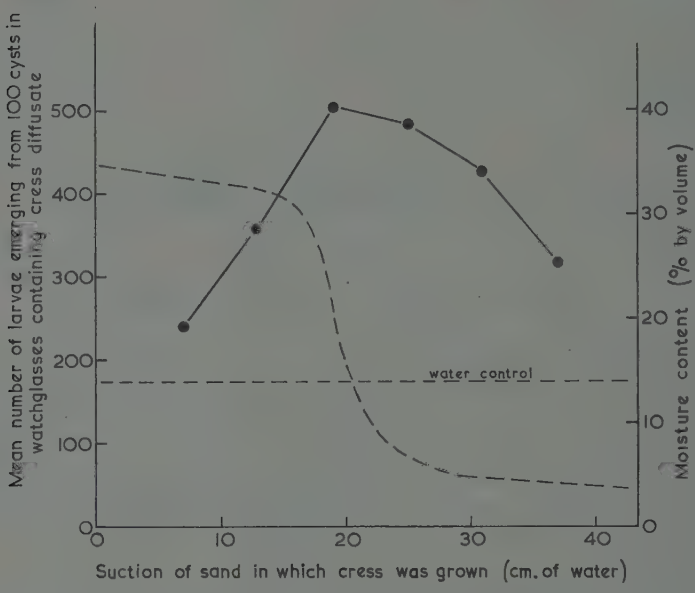

Fig. 4. The influence of suction on the production of hatching factor by cress roots. The dotted curve indicates the moisture characteristic.

low emergence rates *in vitro* rendering the experiment impracticable.

A further factor which would influence emergence is the rate of diffusion of root diffusate. To stimulate hatch, emergence and attraction the factors in root diffusate must travel from the host root to the cyst. To obtain some idea of the rate of diffusion of the hatching factor through a porous medium, beet eelworm cysts were placed in sand of particle size 250-500 μ in polythene tubing at a suction of 15 cm of water, thus most of the pores were full of water. Small quantities of solid diffusate, which was obtained by freeze-drying two litres of aqueous diffu-

sate, were placed at one end of the tubes. Replicate batches of 100 cysts were placed at different distances from the solid diffusate with one batch of cysts per tube. After a week the larvae which had emerged were counted. The results (Fig. 5) show that as the distance between

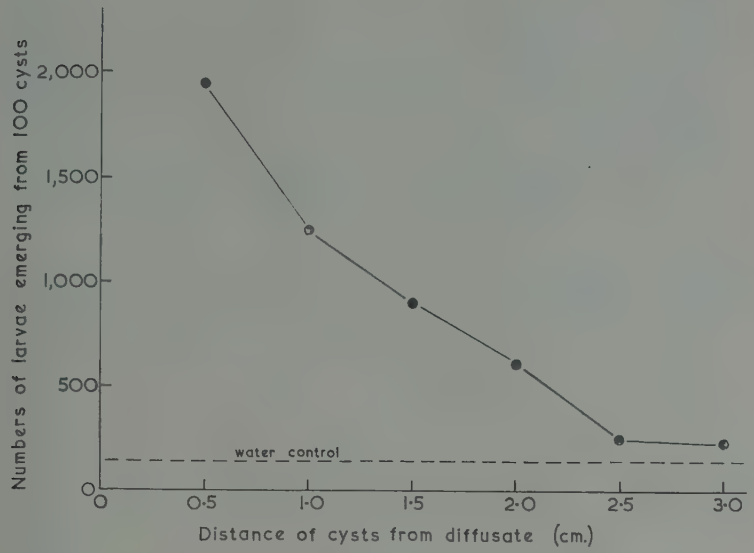

Fig. 5. The rate of larval emergence from cysts of the beet eelworm placed at different distances from a source of diffusate.

cysts and the source of diffusate increases the emergence rate decreases. At 3 cm from the source the emergence rate approaches that in water. Thus at this particular suction the rate of diffusion of the hatching factor is 0.5 cm a day which is of the same order as many other organic and inorganic chemicals diffusing into water. The rate of diffusion decreases as moisture content decreases and, furthermore, there is probably a breakdown of the hatching factor as has been described by Fenwick (1956) for potato root diffusate. This would suggest that there is a range beyond which the diffusate does not have any stimulating properties. No work has been done on the loss of activity with time for either beet or pea diffusate and it remains to be seen whether differences in behaviour of pea root eelworm and beet eelworm are influenced by this factor.

DISCUSSION

On the basis of the experimental results just described it is suggested that beet eelworm and pea root eelworm differ in their behaviour in the soil. Beet eelworm is readily stimulated by root diffusates to give a high percentage emergence but it has a relatively low invasion rate, whereas pea root eelworm has a low rate of emergence but a high invasion rate. This hypothesis appears to be borne out by the results of estimating the numbers of larvae emerging from cysts of pea root eelworm during the spring of 1959 in a field plot under a pea crop. Although the results are not yet complete, it is evident that most of the larvae emerged during the first 7 weeks after sowing and that those which emerged amounted to about 5% of the living egg population of the soil.

Further research is needed to determine emergence and invasion rates more precisely and to see whether the differences in behaviour between these two species of *Heterodera* are due to innate physiological differences between the eelworms themselves or in part at least to the individual characteristics of habit and root growth of their respective host plants. For example, it would be useful to know whether the hatching factor of pea root diffusate is less stable than beet or cress diffusate, and whether the pea root has greater attractant properties than beet, rape or cress for their respective eelworm parasites. Whatever the nature of these differences, the final effect is similar in both species, in that under a host crop an increase in the cyst population occurs until a ceiling level is reached.

ZUSAMMENFASSUNG

Vergleichende Versuche über das Schlüpfmass und die Befallsstärke des Rübennematoden Heterodera schachtii *Schmidt und des Erbsennematoden* Heterodera göttingiana *Liebscher*

Versuche mit Cysten in Sand unter festen Feuchtigkeitsbedingungen zeigten, dass der Rübennematode ein relativ höheres Schlüpfmass hat als der Erbsennematode. Es konnte auch gezeigt werden, dass die Befallsstärke des Erbsennematoden beträchtlich höher liegt als die des Rübennematoden. Diese Unterschiede im Verhalten dürften sich aus den niedrigen Larvenzahlen erklären, die aus Cysten des Erbsennematoden bei in vitro-Versuchen schlüpfen, und anzeigen, dass trotzdem die Populationsvermehrung infolge des hohen Befalls noch kräftig sein kann. Proben von Larven des Erbsennematoden aus einem Erbsenfeld zeigten in der Tat, dass das Schlüpfmass relativ klein ist. Zwei Versuche mit Rübennematoden werden beschrieben, nach denen die Bodenfeuchtigkeit die Produktion des Schlüpffaktors aus Kressewurzeln beeinflussen kann und die Diffusion des Schlüpffaktors durch Sand, der mit Wasser fast gesättigt ist, täglich etwa 0,5 cm beträgt.

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BOOK REVIEWS

Plant Nematodes, their bionomics and control by Jesse R. CHRISTIE, Gainesville, University of Florida, Agricultural Experiment Station. 256 pp, 30 half-tone plates. 34/— (4.75 $ approx.).

The first comprehensive book on plant parasitic nematodes was written by the late Tom GOODEY in 1933 and this was quickly followed in 1934 by FILIPJEV's book in Russian. In 1941, SCHUURMANS STEKHOVEN published a translation and extension of FILIPJEV's book in "the american language" and this, despite its many shortcomings, has been the standard reference work for nearly twenty years. GOFFART's book "Nematoden der Kulturpflanzen Europas", which appeared in 1951, filled a definite need on a more practical level for german readers. For english readers there has been a spate of collected papers from conferences, seminars and courses. Valuable as these may be their coverage and quality are variable and they cannot be regarded as more than stop-gaps until comprehensive text-books appear.

Dr. CHRISTIE's book on "Plant Nematodes, their bionomics and control" fills a need in the english language similar to that of GOFFART's book in german. As is natural the book caters mainly for Florida and the South Eastern region of the United States. European readers may consider the treatment of cyst-forming nematodes somewhat scanty (13 pages) compared for example with the burrowing nematode (16 pages, including 3 pages of host list). Nevertheless the book is on the whole well balanced. After an introductory chapter on nematodes and the symptoms of nematode injury to plants, there follow chapters on control, the root-knot nematodes, cyst nematodes, the burrowing nematode and so on, the groupings partly follow systematic relationships and partly convenience. For each important species of nematode brief, authoritative comments are made on life history and habits, plant injury, hosts, and control. Each chapter ends with a short reference list. Keys, figures and descriptions of nematodes for identification are not given. Dr. Christie expressly prefers the use of common names of nematodes current in the United States rather than latin names because of the instability of the latter. In this way he avoids those controversies in diagnosis and nomenclature which recently have tended to generate heat without much light. The book ends with an appendix containing five useful tables which list respectively crop plants, the nematodes attacking them and the regions in which damage occurs; nematodes, crops attacked and control measures; a summary of times and temperatures required for successful hot-water treatment of infested plants; american common names with latin equivalents; and latin names with their current synonyms.

The book will be especially useful for beginners in Nematology, for agricutural students and advisers in contact with farmers. It is well written in that lucid style which we have come to expect from Dr. Christie. Printing and paper are of good quality and there are thirty half-tone plates mostly illustrating the symptoms of nematode injury to plants.

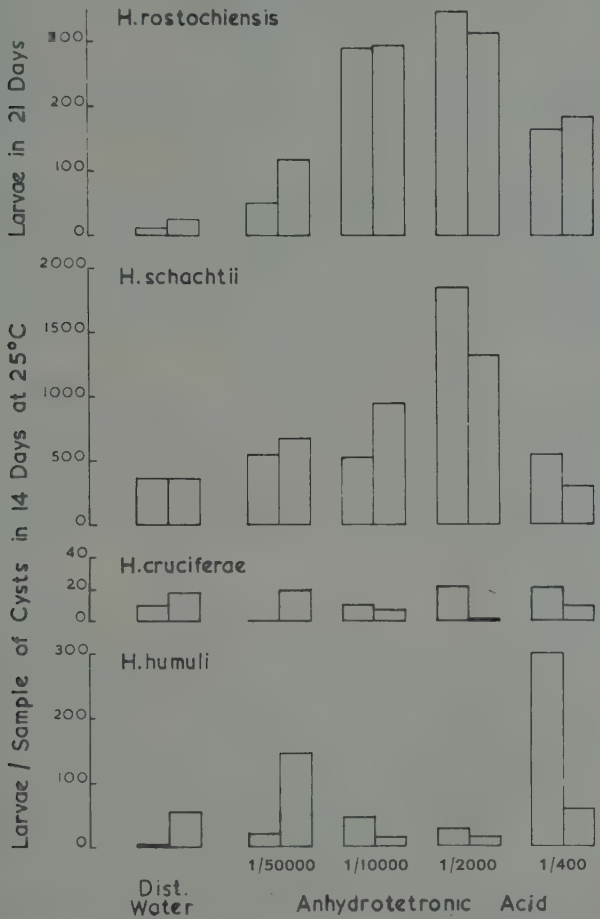
F. G. W. JONES
18th June, 1959

Nematologica 4 (1959) : 237-240. E. J. Brill, Leiden

SCIENTIFIC NOTES

R. D. Winslow [1]), A note on anhydrotetronic acid as a hatching agent of the beet eelworm, *Heterodera schachtii* Schm.

Larval emergence from cysts of the potato root eelworm (*Heterodera rostochiensis* Woll.) is stimulated by anhydrotetronic acid (Calam, Todd & Waring, 1949; Bishop, 1955; Nolte, 1956). To see whether this chemical stimulates the



Larval emergence from duplicate samples of cysts of four species of *Heterodera* incubated in four dilutions of anhydrotetronic acid, and in distilled water controls. Each sample contained 125 cysts, except for cabbage root eelworm where 75 cysts per sample were used. All the acid dilutions were adjusted to pH 3 (approx.) with NaOH or HCl. The pH of the distilled water, which was not adjusted, was 6.0 approx.

[1]) Rothamsted Experimental Station, Harpenden, England.

hatching of other species of *Heterodera*, cysts of four species were incubated in dilutions of the acid, with the results shown in the figure. Peak hatching of beet and potato eelworms occurred in the 1 : 2,000 dilution, but hatching of cabbage eelworm (*H. cruciferae* Franklin) was not stimulated by any of the dilutions used. No conclusion can be drawn from the erratic hatching of the hop eelworm (*H. humuli* Filipjev) population used.

The results suggest that this synthetic hatching agent may be no more related to the potato eelworm hatching factor than to the beet eelworm factor or factors. In fact, anhydrotetronic acid may be only remotely related to the natural agents and it may act in a different way, as already suggested by NOLTE for the potato root eelworm.

Acknowledgement. The sample of anhydrotetronic acid used was supplied by Dr. K. K. Carroll, of the Collip Medical Research Laboratory, University of Western Ontario, London, Ontario, Canada.

ZUSAMMENFASSUNG

Eine Bemerkung über die Anhydrotetronsäure als Schlüpfmittel des Rübennematoden, Heterodera schachtii *Schm.*

Anhydrotetronsäure wirkte in einer Verdünnung von 1 : 2000 schlüpffördernd auf die Larven von *Heterodera schachtii* und *H. rostochiensis,* jedoch nicht bei *H. cruciferae* und anscheinend anders bei *H. humuli.* Sie scheint daher mit den natürlichen schlüpffördernden Stoffen nur entfernt verwandt zu sein.

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NOLTE, H. W. (1956). Beiträge zum Problem der Aktivierung der *Heterodera*-Zysten. *Nematologica* **1**, 72-77.

P. A. A. LOOF [1]), Ueber das Vorkommen von Endotokia matricida bei Tylenchida.

Als Endotokia matricida wird die Erscheinung bezeichnet, dass gelegentlich Eier von normal oviparen Nematodenarten nicht abgelegt werden, sondern sich innerhalb des Muttertieres weiter entwickeln. Nachdem die Larven geschlüpft sind, gelangen sie durch die Wand des Ovars in die Leibeshöhle der Mutter und zerstören deren innere Organe. PAETZOLD (1958) fand in seinen Versuchen mit *Rhabditis (Pellioditis)* sp., dass es den Larven normalerweise gelingt, den abgestorbenen Mutterkörper zu verlassen und sich fortzupflanzen. Aus der Literatur, die von PAETZOLD eingehend berücksichtigt wird, geht hervor, dass Endotokia matricida bei Rhabditiden nicht selten auftritt, ausserhalb dieser Familie jedoch nicht beobachtet wurde.

Im folgenden werden zwei Fälle beschrieben werden, die zeigen, dass auch bei den Tylenchida dieses Phänomen vorkommt, wenn auch unzweifelbar viel seltener.

Der erste Fall bezieht sich auf *Aphelenchoides fragariae* (Ritzema Bos). Im Oktober 1958 wurde in Erdbeeren aus Roosendaal, nahe der belgischen Grenze, in einer Population dieser Art ein Weibchen gefunden, das durch seine eigen-

[1]) Landbouwhogeschool, Wageningen, Nederland.

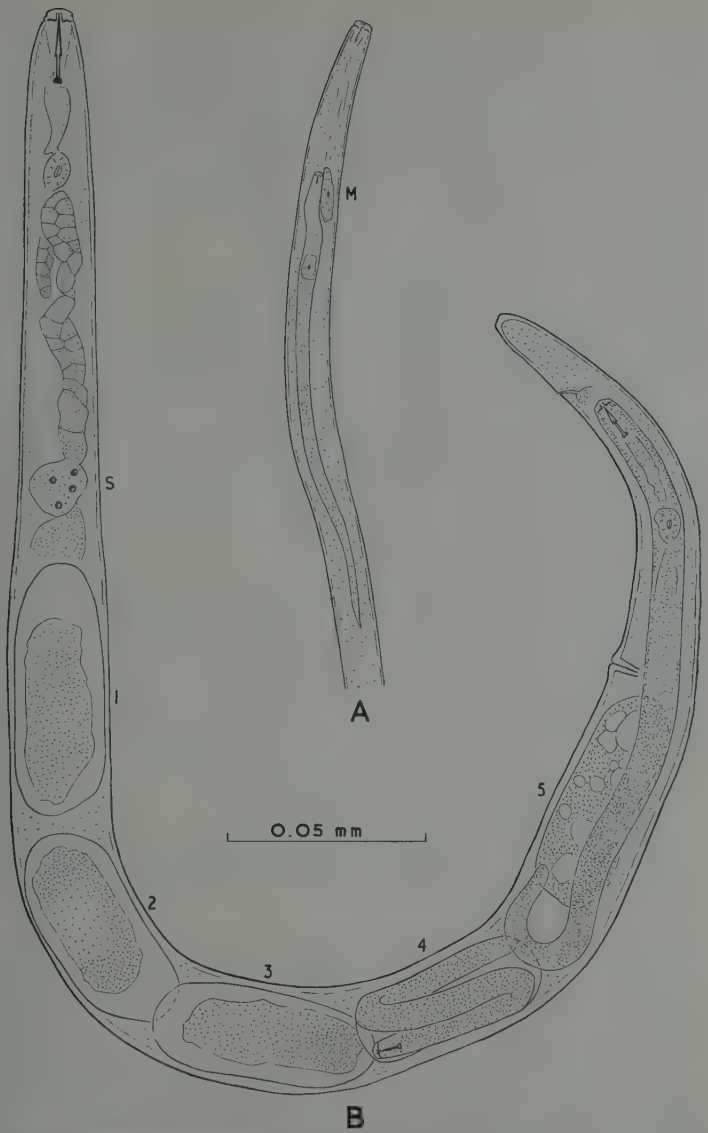

Fig. A. Endotokia matricida bei *Aphelenchoides fragariae*. Vorderende des Weibchens mit Larve. M: Mittelbulbus des Ösophagus.
Fig. B. Dasselbe bei *Pratylenchus coffeae*. Weibchen mit fünf Eiern und einer Larve. S: Spermatheka. 1-5: Eier.

tümliche Bewegungsart auffiel. Es stellte sich heraus, dass dieses Tier abgestorben war und dass sich im Körper eine Larve befand, die in ziemlich lebhafter Bewegung war. Mundstachel und Bulbus waren gut sichtbar. Die Larve hatte den Uterus schon verlassen; der Kopf lag in der Nähe des Afters des Muttertieres. Die Aelchen wurden sofort in eine feuchte Kammer versetzt. Nach vier Tagen befand die Larve sich im vorderen Ende des mütterlichen Körpers, dessen Ösophagus zur Seite gedrängt worden war. Dort verblieb sie neun Tage; ihre Bewegungen wurden seltener und träger. Da die Larve allem Anschein nach sich nicht aus dem Mutterleib herausarbeiten konnte und es zu befürchten war, dass sie sterben und verwesen würde, wurde sie am Ende der genannten Periode fixiert. Die Masse des Muttertieres waren im Präparat; L 498 μ; V 65.9%; a 36,0; c 14,7. Die Länge der Larve betrug 119 μ (Fig. A).

Der zweite Fall betrifft *Pratylenchus coffeae* (Zimmermann). In einer von Dr. Yokoo aus Japan erhaltenen, fixierten Population wurde ein Weibchen aufgefunden, das im Körper fünf Eier und eine freie Larve trug. Die Masse dieses Tieres (ebenfalls im Präparat gemessen) waren: L 527 μ; V 79,8%; a 20,7; c 21,8; G 71,7%. Die freie Larve mass 176 μ und die Eier: Nr. 1 62 x 22 μ; Nr. 2 52 x 23 μ; Nr. 3 53 x 23 μ; Nr. 4 48 x 20 μ und Nr. 5 54 x 18 μ (Fig. B). In Nr. 1, 2 und 3 hatte sich der Inhalt von der Eihülle zurückgezogen, und das Aussehen dieser Eier legte die Vermutung nahe, dass die Furchung schon ziemlich weit fortgeschritten war (vergleiche Besprechung und Abbildungen der Eientwicklung bei Zimmermann, 1898). Deutliche Zellgrenzen waren nicht zu beobachten. Ei Nr. 4 enthielt eine schon nahezu voll entwickelte Larve, während Nr. 5 wohl die leere Hülle darstellt, aus der die freie Larve geschlüpft ist.

Infolge des Wachstums der grossen Anzahl von Eiern war das Ovar deformiert und so weit hinaufgeschoben, dass sein Anfang, wenn es nicht umgebogen wäre, sich nahe dem Kopfende des Tieres befunden haben würde. Auch die Spermatheka war weit cephalad gerückt.

Intrauterine Geburten sind nach Maupas (1899) verbreitet bei allen *Rhabditis*-Arten mit grosser Eizahl und rascher Fortpflanzung. *Pratylenchus* vermehrt sich langsam; normalerweise findet sich nur ein reifes Ei im Uterus. Es ist aber bemerkenswert, dass gerade bei *P. coffeae* die intrauterinen Eier oft Larven enthalten, wie schon von Cobb (1920) bei *Tylenchus mahogani* = *Pratylenchus coffeae* beobachtet würde. Bei anderen *Pratylenchus*-Arten fängt die Furchung nach der Eiablage an oder es werden doch nur die ersten Teilungen im Uterus durchlaufen.

Ueber die Fortpflanzungsgeschwindigkeit von *Aphelenchoides fragariae* wurden keine Angaben gefunden. Es ist aber bekannt, dass die verwandte Art *A. ritzemabosi* (Schwartz) sich stark und schnell vermehren kann (Siehe Filipjev & Schuurmans Stekhoven, 1941).

SUMMARY

Endotokia matricida is described in *Aphelenchoides fragariae* and *Pratylenchus coffeae*. This phenomenon had not yet been observed outside the family Rhabditidae.

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ON THE NUMBER OF GENERATIONS OF BEET EELWORM, *HETERODERA SCHACHTII* SCHMIDT, PRODUCED IN A YEAR

BY

J. J. DUGGAN
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While testing the host-range of beet eelworm (*H. schachtii* Schmidt, 1871), two distinct "flushes" of white cysts appeared on the roots of mangolds during the season. The mangolds had been sown in tubes of infected soil (DUGGAN 1957), in April and the flushes of white cysts appeared in June and in September.

Following the September examination of the plant roots, the tubes were re-plunged in peat-moss in the field and were examined at fortnightly intervals. A third "flush" of white cysts appeared on the roots of some of the plants the following January, indicating that the parasite had been active over the winter months. This appearance of white cysts in January could be attributed to one or both of the following causes : —

(a) The contents of cysts which had not become completely emptied before September, may have emerged later in the year, invaded the roots of the mangolds and given rise to the cysts which appeared in January.

(b) The eggs in the white cysts which appeared on the roots in September may have matured and hatched and the larvae may have invaded the mangold roots and produced the cysts which were observed in January.

The latter explanation, if correct, would mean that more than one generation of beet eelworm is produced in a year on mangolds. TRIFFITT (1930) concluded that, in England, only one generation per year was possible and she attributed the occurrence of infection throughout the summer to the fact that old cysts become emptied of their contents gradually and not in a single hatch. JONES (1950), observed that two and a half to three generations (depending on the season), were possible in an average crop of sugar beet which occupies the land from April to

1) Now Agricultural Institute, Dublin.

November. He further observed that "for a sugar-beet seed crop for which mother seed is normally sown in July one and a half to two generations would be completed in the steckling bed before planting out in March of the following year, while a further two generations would be possible in the seedling stage between March and August". FRANKLIN (1951) thought that a limited second generation might be produced but that there was no likelihood of a third generation.

To obtain information on the number of generations which could be produced in a year under Irish conditions the writer carried out the following experiment.

DETAILS AND METHODS

One hundred and fifty test-tubes were filled with soil infected with *H. schachtii*. On the 15 April 1957 single mangold seedlings, which had been raised in sterilised seed compost, were planted in the tubes which were then plunged in peat-moss in the field.

These tubes were lifted in batches of fifty after periods of 6, 8 and 10 weeks. Thus, the first lot of tubes was lifted on 27 May, the second lot on 10 June and the remaining lot on 24 June. When cysts were observed on the roots, the mass of soil and roots was removed, unbroken, from the tube by inserting a jet of water through the drainage hole and the cysts were then picked off. Cysts thus obtained were used to inoculate tubes of sterilised soil, thirty cysts being added to each tube, which was then planted with mangold seedlings and plunged as before. Twenty tubes were inoculated with the cysts produced after 8 weeks and thirty tubes with the cysts produced after 10 weeks.

All eighty tubes were examined periodically and a "flush" of newly-formed cysts was observed early in September. These cysts were recovered as described and, on 27 September, they were used to inoculate twenty tubes of sterilised soil, thirty cysts being added to each tube. The tubes were planted with mangold seedlings and plunged once more.

From 8 November, the tubes were examined at fortnightly intervals. On 8 January white cysts were observed on the roots of plants in six of the tubes.

DISCUSSION OF RESULTS [1])

In the above experiment, a third generation of cysts was produced

1) In the following discussion, the terms "6-week-old cysts", "8 week-old cysts" and "10 week-old cysts", are used to refer to cysts formed after 6, 8 and 10 weeks, respectively.

in January 1958 i.e. within a year of the first sowing (April 1957). However, the three generations were not produced within the normal growing season for mangolds in the field as this crop is usually lifted in November.

The second generation of cysts was produced abundantly in the tubes inoculated with the 8 and 10 week-old cysts. A few second-generation cysts were produced in two of the tubes inoculated with the 6 week-old cysts but no infection was observed in the remaining eighteen tubes of this series. This indicates that eggs within the white "cysts" were not fully developed when they were picked off the roots after six weeks or that they were more easily damaged, at this stage of development, in the "picking off" process. The few cases of infection which occurred might be because those cysts which were in a cluster on a rootlet were not picked off individually but the rootlet to which they were attached was cut off with the cysts intact.

While the tubes inoculated with the 8-week-old cysts were planted a fortnight earlier than those inoculated with the 10 week-old cysts, the new generation of white cysts did not appear in either set of tubes until early September despite the fact that one lot of tubes had been planted on 12 June and the other on 26 June. While cysts formed in June were capable of producing a second generation within the season the eggs within them evidently required a period of at least four weeks in which to mature before they were capable of hatching. Jones (1956) observed that newly produced eggs require a period of maturation before they are ready to hatch.

The population-changes which occur with each generation require more detailed study. In the above experiment, the cyst-density on the roots of the plants was greatest for first and second-generation cysts. Comparatively few cysts were formed in the third generation indicating that eggs from most of the second-generation cysts which had been used to inoculate the sterilised soil either failed to hatch or, (if they hatched and invaded the roots) failed to develop and produce cysts. These observations indicate that populations of *H. schachtii* are built up, mainly by the first and second generations. It is conceivable that more larvae invading roots in September and November may develop by, say, April rather than January because, as Jones (1950) observes, the rate of cyst-development slows down as soil temperatures fall in the autumn and it becomes negligible in the winter months from November to March.

Had the third generation been as prolific as the first and second,

leaving a host-crop such as swedes certain Brassica seed crops, beet, fodderbeet and mangold seed crops in infected soil over the winter could produce a dangerous degree of infestation in a short time, though JONES (1956-57), observed that the ceiling level produced by a host crop is not greatly influenced by length of vegetative period. Although it has been shown experimentally that *H. schachtii* is capable of producing three generations in a year, detailed studies of population changes still need to be done on host crops such as swedes, or the seed crops referred to above which are left in the ground long enough for the production of a third generation of cysts.

ZUSAMMENFASSUNG

Über die Anzahl der Generationen des Rübennematoden (Heterodera schachtii Schmidt) *innerhalb eines Jahres*

Hundertfünfzig Röhrchen wurden mit Boden gefüllt, der mit *Heterodera schachtii* infiziert war. Im April wurden Mangoldsämlinge in die Röhrchen gepflanzt, die sich an den Wurzeln bildenden Zysten abgenommen und zur Infektion einer weiteren Reihe von Röhrchen benutzt. Die Zysten der zweiten Generation, die sich an den Mangoldwurzeln dieser Reihe entwickelten, wurden für einen dritten Röhrchensatz zur Infektion verwendet. Im folgenden Januar konnten Zysten der dritten Generation an einigen Pflanzen beobachtet werden. In diesem Versuch war die Zystendichte an den Wurzeln der Pflanzen bei der ersten und zweiten Zystengeneration am grössten. Im Vergleich hierzu bildeten sich nur einige Zysten der dritten Generation. Die Beobachtungen zeigen, dass Populationen von *Heterodera schachtii* hauptsächlich von der ersten und zweiten Generation aufgebaut werden.

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FURTHER OBSERVATIONS ON SOME FACTORS INFLUENCING THE EMERGENCE OF LARVAE FROM CYSTS OF THE BEET EELWORM *HETERODERA SCHACHTII* SCHMIDT

BY

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Previous experiments (WALLACE 1954, 1955a) have shown that the rate of larval emergence from cysts is related to the pressure deficiency or suction in sand or soil. There does not appear to be a direct effect of suction on emergence, because experiments with a range of sand particle sizes have indicated that the optimum suction for emergence approximates to the stage where most of the water has been removed from the pore spaces and is confined to the points of contact of particles. Soil at this particular moisture content is said to be at field capacity. Emergence rate is greatly influenced by oxygen concentration (WALLACE 1955b) and it was deduced from this that low emergence rates at low suctions were due to lack of aeration. Observations on emergence of larvae from cysts have shown that at high suctions the surface tension forces of the water films at the cyst openings inhibit emergence. It is the object of this paper to consider these factors further and, on the basis of the results obtained, to suggest how the rate of larval emergence from cysts may be influenced by environmental conditions in the soil.

Sand was used throughout the experiments and suctions were controlled by methods described previously (WALLACE 1954, 1955a). A temperature of 24°C was used for all experiments.

RESULTS

In earlier experiments the influence of suction on rate of emergence in sand was determined by counting the larvae which had emerged from a given number of cysts in a given time. The form of the emergence curve with time for a particular set of cysts has not been deter-

mined as it was in experiments *in vitro* (Fenwick 1950, Winslow 1954). To investigate this, replicate batches of 100 cysts were placed in each of three suctions in polythene tubes containing a sand fraction of 250-500 μ particle diameter. At weekly intervals the sand, cysts and emerged larvae were washed into a specimen tube. When the sand had settled the larvae and cysts were decanted into a petri dish for counting.

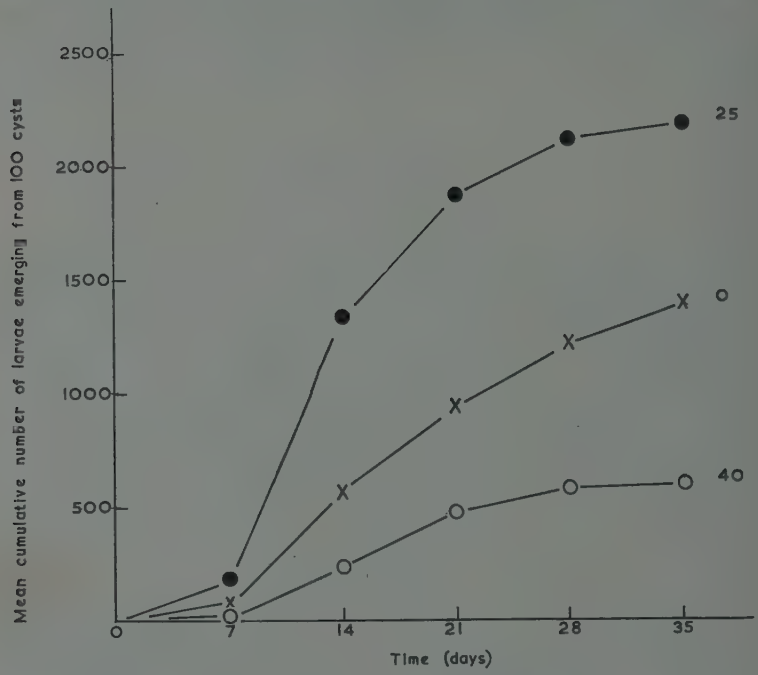

Fig. 1. Emergence of larvae from cysts in sand at three different suctions, 0, 25 and 40 cm of water. Each point is the mean of four replicates.

By decanting three times, all the cysts and over 95% of the larvae could be recovered from each tube. The cysts were then replaced in sand in polythene tubing and the suction was again adjusted. Three suctions were employed and the results are given in Fig. 1. The emergence curves at each suction are sigmoidal and resemble those with experiments in watchglasses. The relatively high emergence rate at 25 cm of water suction corresponds to the point on the moisture characteristic where most of the water has been removed from the pore spaces and is the optimum suction for emergence in sand of this parti-

cular size (Wallace 1956a). The low emergence rates at 0 and 40 cm water suction agree with previous results that, under saturated conditions and in conditions where there is little moisture, the rate of emergence is low.

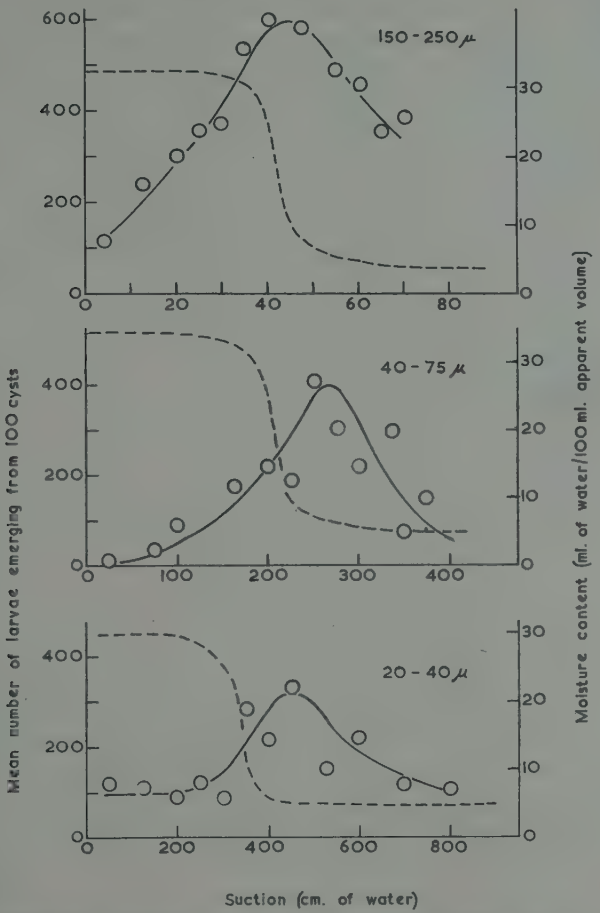

Fig. 2. Emergence of larvae from cysts in 3 different particle sizes of sand at different suctions. Each point is the mean of two replicates. The dotted lines indicate the moisture characteristics i.e. the relationship between suction and moisture content.

The suction at which maximum emergence occurs is related to particle size (Wallace 1955a), corresponding to the point on the moisture

characteristic where most of the water has been removed from the pore spaces. It has been stated (WALLACE 1956b) that there is a limiting suction of 150-200 cm of water above which emergence probably does not occur. To test this hypothesis, sand of particle size much less than that previously used was employed. The emergence curve over a range of suctions in each of these sand fractions was determined and the results are expressed in Fig. 2. In all three sand fractions there was an optimum suction for emergence. Thus, in the 40-75 μ sand the op-

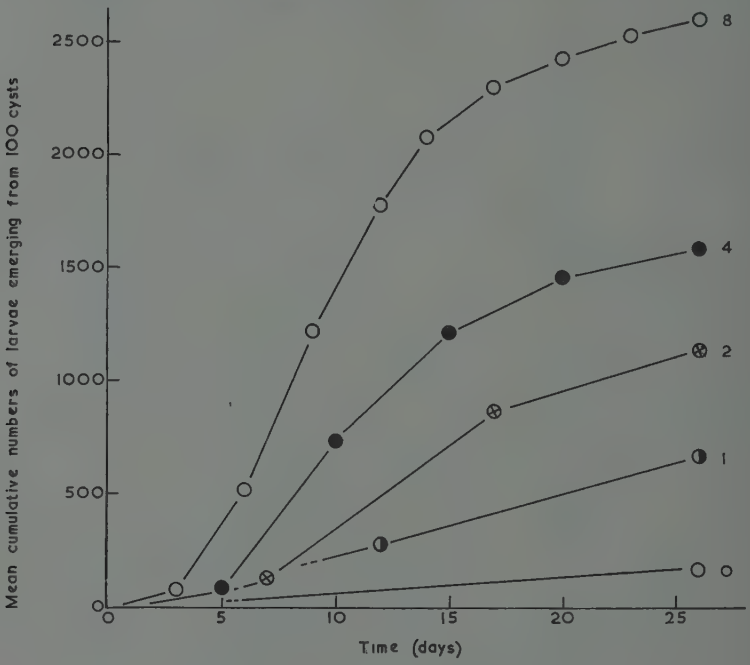

Fig. 3. Emergence of larvae from cysts in sand under controlled moisture conditions with a different number of changes of sand and water during 26 days. Each point is the mean of four replicates.

timum suction was 250 cm of water and in the 20-40 μ fraction it was about 500 cm of water. The results show that the hypothesis of a limiting suction for emergence is untenable and that the water distribution in the sand is the limiting factor whatever the particle size. The results also indicate that the maximum emergence in the 150-250 μ fraction is about twice that in the 20-40 μ fraction confirming a previous

opinion (WALLACE 1956c) that emergence rate decreases as particle size decreases.

When larvae hatch and emerge from the cyst it seems possible that there will be an accumulation of excretory products in the vicinity of the cyst which may inhibit further hatch. Batches of 100 cysts in polythene tubes containing sand of 250-500 μ particle size at a suction of 25 cm of water were studied over a period of 26 days. Using four fold replication with batches of 100 cysts, five treatments were given, the cysts being removed from the sand and replaced in fresh sand at the same suction 0, 1, 2, 4 or 8 times during the 26 days. The results (Fig. 3) show that the rate of emergence increased with the number of changes. It is suggested that when the cysts were placed in fresh surroundings inhibitory substances were removed and further emergence then took place. The nature of this inhibitory substance is not known but the fact that the rate of emergence can be increased under saturated conditions by decreasing the depth of the fluid in which they are immersed (DEN OUDEN 1954, SHEPHERD 1959, WALLACE 1956a) suggests that the substance diffuses readily into the atmosphere. It seems possible, therefore, that carbon dioxide may be the inhibitor. GILLARD, D'HERDE & VAN DEN BRANDE (1958) have shown that carbon dioxide does, in fact, inhibit emergence and SHEPHERD (*in litt.*) has found that, with equal oxygen concentration but varying carbon dioxide concentration, the emergence rate varies inversely as the carbon dioxide concentration.

By placing cysts in a single layer of sand in water containing beet root diffusate it was possible to observe larvae emerging from the cysts. By removing the water between the particles with filter paper, three different water regimes were obtained: (1) pores full of water; (2) most of the pores empty of water but with large amounts at the points of contact of particles; (3) pores empty and little water at the points of contact. By watching about 300 cysts under the microscope it was possible to determine the minimum, maximum and mean times for twenty individuals to emerge from different cysts in each of these three moisture conditions. The results were (1) 3-10 minutes with a mean of 7.3 minutes, (2) 0.1-0.5 minutes, mean 0.3 minutes, (3) 15-30 minutes, mean 24 minutes. It was apparent that speed of emergence was highest in the second water regime, where there were films outside the cyst openings in which the eelworms could progress. The relationship between film thickness and speed of locomotion has been described by WALLACE (1958). The optimum conditions for such locomotion occurred in the intermediate moisture content in the sand. Where the sand

was saturated movement of eelworms out of the cyst was slow because *H. schachtii,* unlike other more active species, is unable to swim in water because the propulsive forces generated by the eelworm are low. In sand where there is very little water, movement is also slow because the water films are so thin that the larva is pulled down on to the soil particle by surface tension and cannot overcome the relatively high friction forces opposing motion. The observations explain the similarity in the curves for emergence and mobility (WALLACE 1955a, 1958a). In fact movement out of cysts and movement through soil and sand pores are fundamentally the same phenomenon and are subject to the same factors influencing locomotion.

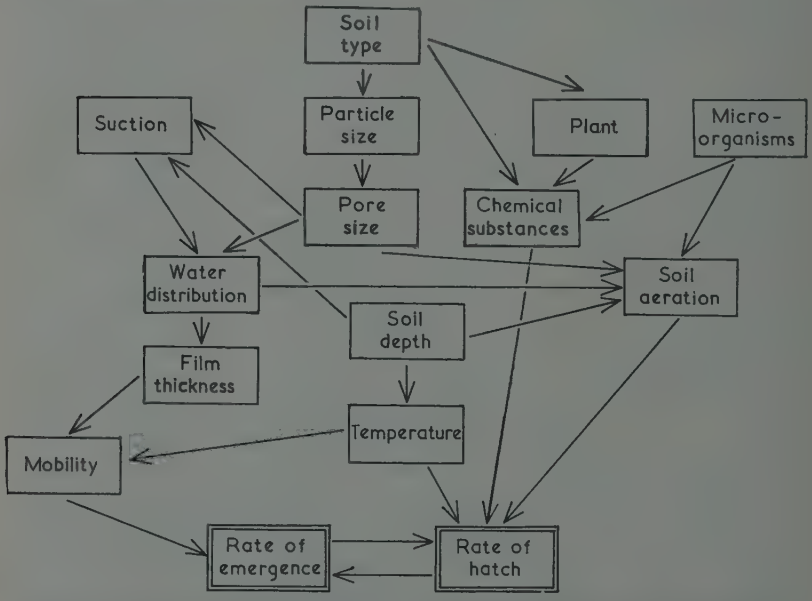

Fig. 4. The inter-relationship between soil environmental factors and larval hatch and emergence from cysts.

DISCUSSION

The rate at which eggs hatch within the cyst determines the rate at which larvae emerge from the cyst. The converse is also true because the presence of active larvae within the cyst inhibits further egg hatch (WALLACE 1955b) and the same has been observed for potato root eelworm (ELLENBY 1946, HESLING 1959, ONIONS 1955), so the rate at which larvae emerge determines the rate at which further eggs hatch.

The factors in the soil which influence the rate of larval emergence from the cyst are closely inter-related. Thus, if soil moisture alone is considered, it is apparent that this will control both aeration and the thickness of the water films which in turn influence both hatch and emergence, these themselves being inter-dependent. When other factors are considered the picture becomes confused. Fig. 4 is a diagram showing hypothetically the relationship between emergence and the edaphic and biotic factors in the soil. The diagram is incomplete and oversimplified but it gives some impression of the kind of situation which exists in the soil.

The arguments developed so far refer only to beet eelworm. The few experiments of a similar nature which have been done with other species suggest that it would be unwise to apply these principles to the whole genus (WALLACE 1958b, SHEPHERD & WALLACE 1959). The influence of host plants on emergence of beet eelworm has been studied by WINSLOW (1954, 1957), but root diffusates only appear to superimpose an increased rate of emergence on the pattern already existing between emergence and the physical factors of the environment.

ZUSAMMENFASSUNG

Weitere Beobachtungen über einige Faktoren, die das Ausschlüpfen der Larven des Rübennematoden Heterodera schachtii *Schmidt beeinflussen*

Die Form der Schlüpfkurven für Rübennematoden in Sand bei festgelegtem Feuchtigkeitsgehalt ist ähnlich derjenigen bei Versuchen in vitro. Im Boden besteht eine optimale Saugkraft für das Schlüpfen und diese bezieht sich auf die Teilchengrösse. Je kleiner die Teilchengrösse, desto höher ist die optimale Saugkraft. Es scheint keine begrenzende Saugkraft für das Schlüpfen zu bestehen, wie früher angegeben wurde. Bei optimaler Saugkraft sinkt die Schlüpfhöhe mit der Partikelgrösse. Es wird angenommen, dass bei fortschreitendem Larvenschlüpfen eine Anreicherung von Kohlendioxyd eintritt, die eine weitere Entwicklung und ein weiteres Schlüpfen verhindert. Schlüpfen aus den Zysten und Bewegung durch die Bodenporen sind grundsätzlich das gleiche Phänomen und denselben Faktoren unterworfen, die die Bewegung beeinflussen. Eine Hypothese wurde aufgestellt, um zu zeigen, wie das Schlüpfen durch die Bodenumgebung beeinflusst wird.

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THE INVASION AND DEVELOPMENT OF SOME SPECIES OF *HETERODERA* IN PLANTS OF DIFFERENT HOST STATUS

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This paper attempts to show the relative efficiency of certain species in the families Chenopodiaceae and Cruciferae as hosts of beet eelworm, *Heterodera schachtii* Schmidt, and to follow the development of larvae in their roots under comparable conditions. Invasion has also been examined in plants which do not produce an active hatching factor, using larvae of three species of *Heterodera*.

The efficiency of hosts of cyst-forming eelworms has mostly been evaluated on the basis of cyst production (Hijner, 1951), although a more detailed study of the development of larvae within roots has been made in certain plants (Doncaster, 1957; Apel & Kämpfe, 1957a, b; Golden, 1958).

MATERIALS AND METHODS

In the experiment on host efficiency, the plants used were: Chenopodiaceae; sugar beet (*Beta vulgaris*), *B. trigyna*, *B. maritima*, *B. webbiana*, *B. procumbens*, *B. patellaris*, fat hen (*Chenopodium album*): Cruciferae, cabbage (*Brassica oleracea* var. *capitata*), cress (*Lepidium sativum*), radish (*Raphanus sativus*), charlock (*Brassica sinapis*), stock (*Matthiola incana*), dame's violet (*Hesperis matronalis*).

Seedlings were grown singly in potting compost in 2½ in. diam. pots. To ensure control of inoculum levels, hatched larvae were used in preference to cysts (Shepherd, 1958) and in a further attempt to reduce variability, the invasion period was limited to one week. A preliminary experiment was made using *Hesperis matronalis* and sugar beet, to see whether transplanting infested plants one week after inoculation into uninfested soil would affect the natural course of development of the larvae within the roots of susceptible and resistant plants.

Invasion and development were traced in both transplanted and untransplanted seedlings of the two species and no statistically significant difference was observed. Thereafter transplanting was used for the host-efficiency test.

Twenty plants of each species were inoculated with 2,000 larvae of *H. schachtii* and, one week later, the roots were gently washed free from soil and transplanted into uninfested potting compost in 4 in. diam. pots. Four plants of each species were removed at weekly intervals from the day of inoculation for five weeks, and the root systems fixed and preserved in formol-acetic-alcohol. The numbers of larvae at the various stages of development in the whole root system were estimated by staining the roots in acid fuchsin and macerating them in a Waring blender (GOODEY, 1957). Aliquots of the resulting suspension were counted, but when the number of worms was low the whole suspension was examined. A stereoscopic microscope was used for counting, and the estimation of the larval stages was based on those illustrated by RASKI (1950). An attempt was also made to distinguish between larvae that were living or dead before the roots were fixed, by assuming that dead ones were those that were almost empty. Often, only the cuticle remained. The counts on which the graphs in Figs. 1-5 are based are the totals of "living" and "dead" individuals.

TABLE I

Status of plants used to compare invasion in hosts, resistant plants producing a hatching factor and plants which do not produce a hatching factor, in relation to three species of Heterodera

Plant	Host status		
	H. schachtii	*H. rostochiensis*	*H. göttingiana*
Beet	***	*	*
Pea	*	*	†
Tomato	*	***	*
Clover	*	*	*
Aster	*	*	—
Oats	*	*	—
Grass	*	—	—
Hesperis	**	—	—
Cabbage	—	*	—
Solanum nigrum	—	**	—

— not tested
*** host
** non-host, but hatching factor produced
* non-host, no hatching factor produced
† host, but hatching stimulus weak or absent

To study invasion in plants which do not produce a hatching factor, larvae of *H. schachtii, H. rostochiensis* Woll. and *H. göttingiana* Lieb. were used. The method was essentially similar to that described above, with an inoculum of 2,000 larvae and fourfold replication. With *H. göttingiana,* however, twofold replication was used, because of the difficulty of obtaining hatched larvae in quantity. The number of larvae in the roots was estimated after 2 weeks. Table I shows the status of the plants tested with reference to each nematode species.

The effect of the presence of a host plant on invasion in a plant not producing a hatching factor was also examined. Pea and sugar beet plants were grown separately and together in 4 in. diam. pots and 2,000 larvae of *H. schachtii* added. The larvae in the root systems of each plant were then counted. The replication was fourfold and either two peas, two sugar beets, or one pea and one sugar beet were grown per pot. The plants were left for two weeks after inoculation, then the root systems were washed free from soil, after which they were easily disentangled from each other.

RESULTS

To simplify the presentation of results (Figures 1-5) from the experiment on the status of plants in the Chenopodiaceae and Cruciferae as hosts of beet eelworm, the plants used have been separated arbitrarily into two groups within each of the two families. All the plants tested were invaded, the invasion rates being shown by the numbers present in the roots at the end of the first week in Fig. 1. Third stage larvae (Fig. 3) were found in the roots of all the plant species, although sometimes in small numbers. No fourth stage larvae (Fig. 4) were found in *B. procumbens* or *B. webbiana* and, although a few males were produced in *B. patellaris,* no fourth stage larvae were found in this species either, probably because second week samples of *B. patellaris* were not available because of poor germination. Fifth week samples of *B. patellaris* were also omitted for the same reason. Among the Cruciferae, the fourth stage was not reached in *Hesperis* or *Matthiola.* No males (Fig. 5a) were observed in *B. procumbens, Hesperis* and *Matthiola,* and only one in *B. webbiana.* No females (Fig. 5b) were found in *B. procumbens, B. patellaris, Hesperis* and *Matthiola,* very few in *C. album,* radish and charlock and only one in *B. webbiana.* Two or three cysts have previously been found on *B. patellaris* (SHEPHERD, 1959).

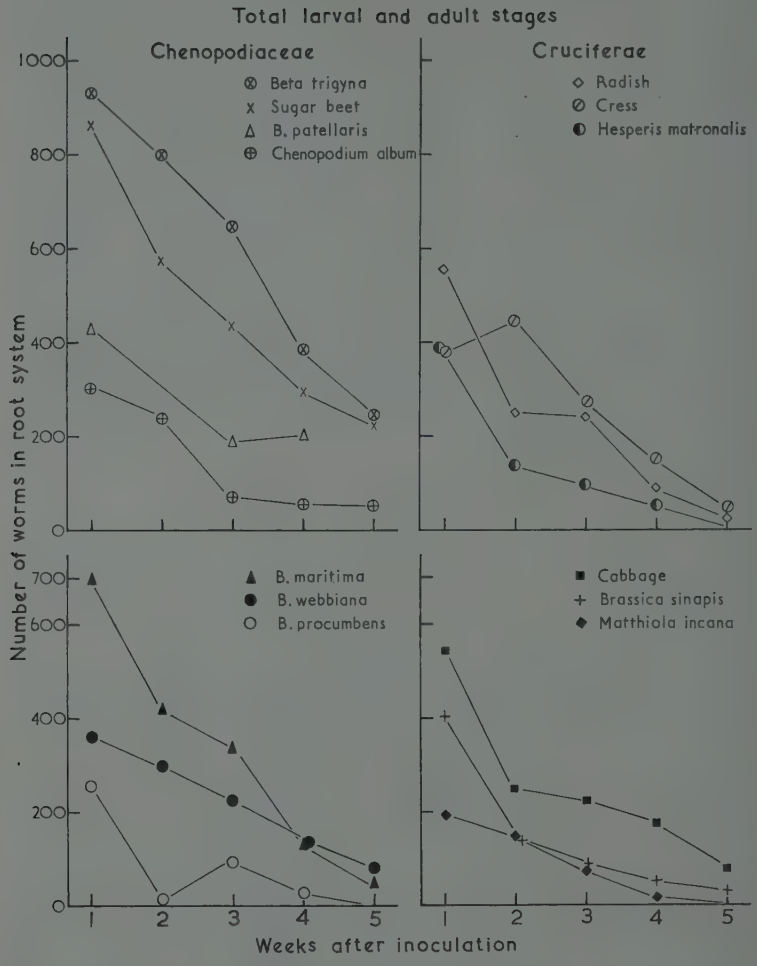

Fig. 1. Total numbers of adult and larval stages of *Heterodera schachtii* present in the root systems of various species of Chenopodiaceae and Cruciferae during five weeks after inoculation. All points are means of four replicates. The first week points represent invasion levels.

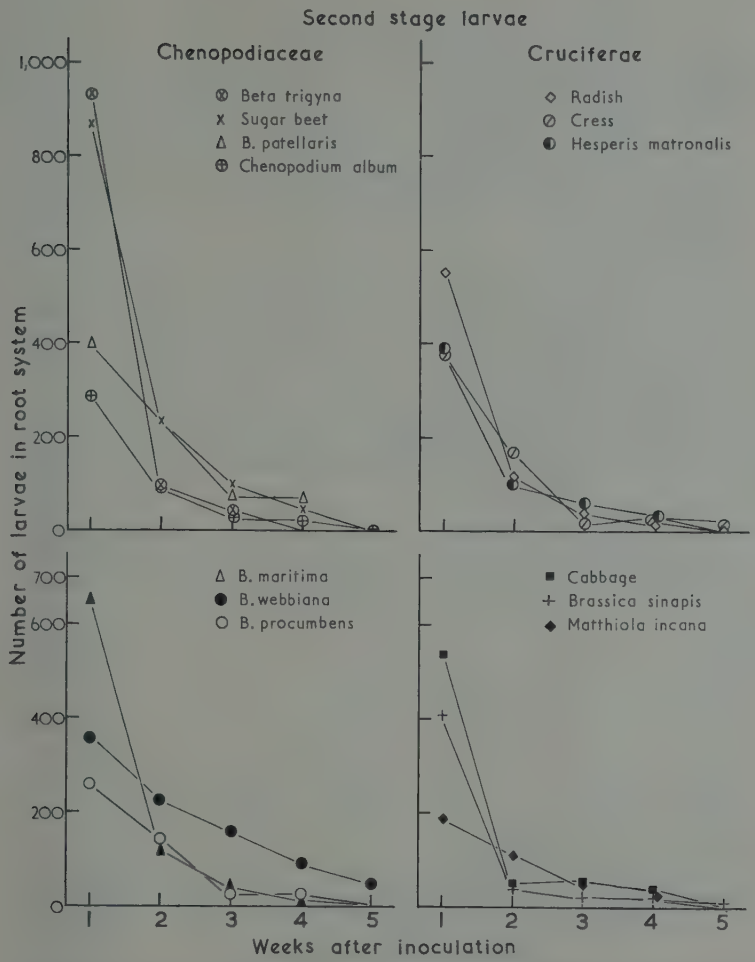

Fig. 2. Numbers of second stage larvae of *Heterodera schachtii* present in the root systems of various species of Chenopodiaceae and Cruciferae during five weeks after inoculation. All points are means of four replicates.

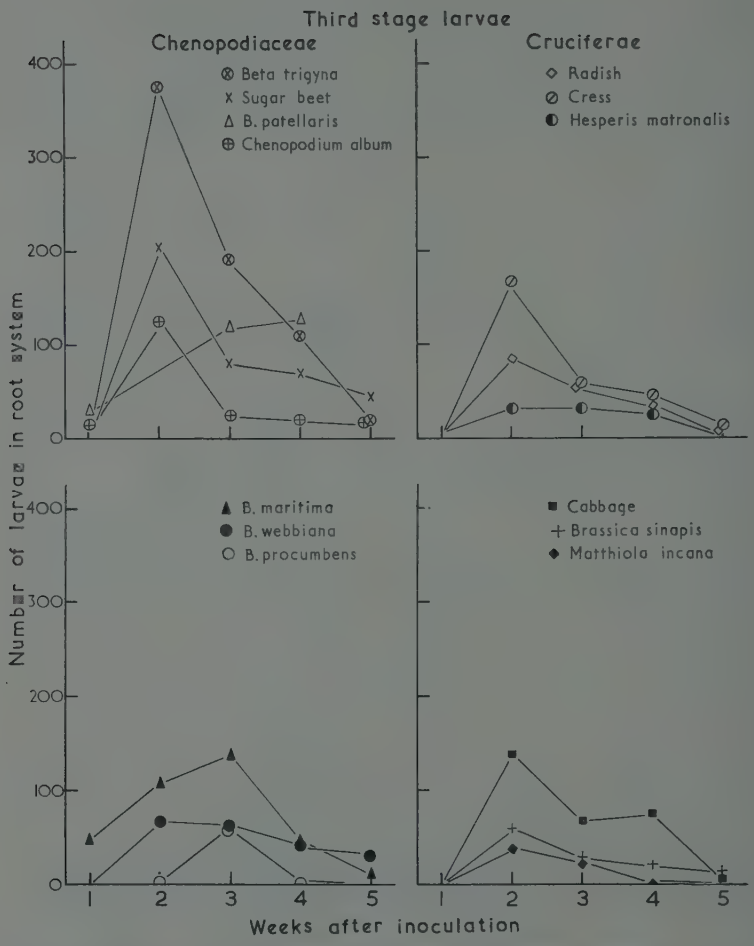

Fig. 3. Numbers of third stage larvae of *Heterodera schachtii* present in the root systems of various species of Chenopodiaceae and Cruciferae during five weeks after inoculation. All points are means of four replicates.

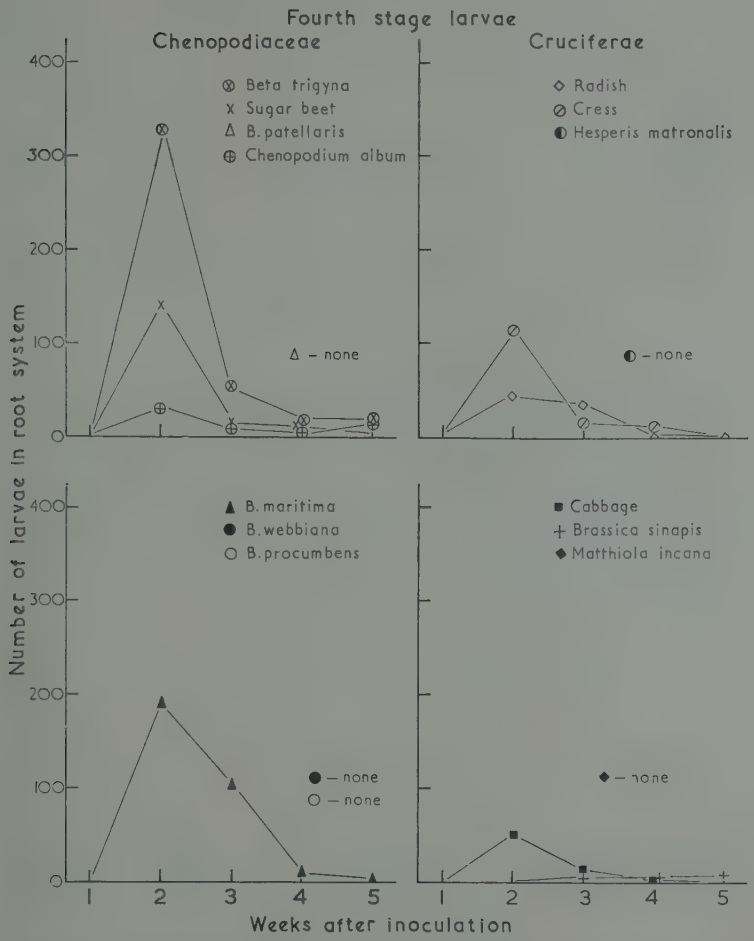

Fig. 4. Numbers of fourth stage larvae of *Heterodera schachtii* present in the root systems of various species of Chenopodiaceae and Cruciferae during five weeks after inoculation. All points are means of four replicates.

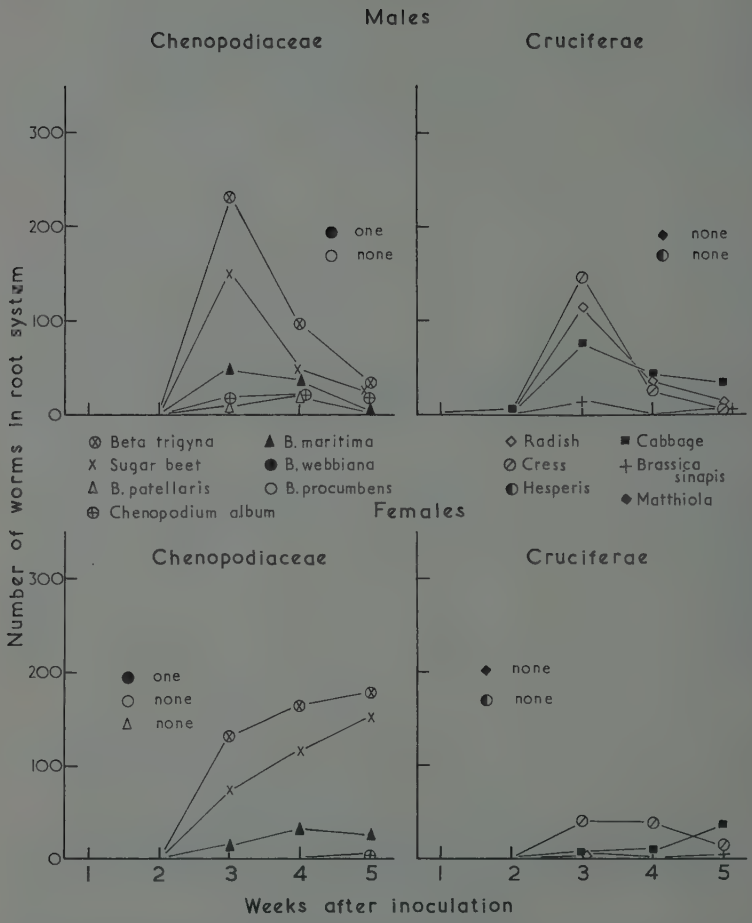

Fig. 5. Numbers of males and females of *Heterodera schachtii* present in the root systems of various species of Chenopodiaceae and Cruciferae during five weeks after inoculation. All points are means of four replicates.

TABLE II

Percentage of nematodes in each stage of development which were apparently dead prior to fixing the roots

Plant	Larval stage			Male	Female	Total
	2nd	3rd	4th			
Chenopodiaceae						
Beta trigyna	28	65	9	6	7	28
Sugar beet	30	53	2	0	11	26
B. maritima	17	59	23	3	0	26
B. patellaris	60	83	—	0	—	66
B. webbiana	80	95	—	—	—	93
B. procumbens	83	68	—	—	—	75
Chenopodium album	12	14	6	2	0	11
Cruciferae						
Cabbage	31	54	0	0	0	33
Cress	15	43	2	0	0	16
Radish	25	26	9	2	0	18
Hesperis matronalis	15	26	—	—	—	17
Brassica sinapis	10	17	19	0	0	11
Matthiola incana	29	8	—	—	—	26

The highest proportion of apparently dead larvae occurred in the second and third stages (Table II). The numbers of "dead" observed by no means account for the fall in numbers from week to week. The larvae seem to disappear very rapidly in the roots, presumably either by autolysis or by bacterial decay from a flora taken in during invasion. The cuticle itself also disappears rapidly, for when present, the empty cuticles were easily detected and recorded amongst the dead.

The following observations can be made from the data illustrated in Figs. 1-5 and Table II.

1. Invasion rate varies with the species of host plant.
2. The susceptible species of Chenopodiaceae are better hosts than the susceptible species of Cruciferae.
3. *B. trigyna* is a better host than sugar beet, and both of these are more efficient than *B. maritima*.
4. *B. patellaris* falls between the susceptible and the more resistant species of *Beta* in that a small proportion of the larvae which invade develop into males (see also SHEPHERD, 1957).
5. The death rate in the roots may be high, even in susceptible species.

Table III illustrates the results of adding larvae to plants which do not produce a hatching factor and are considered to be non-hosts of the respective species of *Heterodera* used. All the plants were invaded,

although some only slightly. However, even the host controls were not very heavily invaded in this experiment, probably because of high temperatures just after inoculation. On the whole, more larvae of *H. rostochiensis* invaded the non-host roots than did larvae of *H. schachtii* and *H. göttingiana.*

TABLE III

Comparison of invasion by three species of Heterodera *in roots of hosts, resistant plants producing a hatching factor and plants not producing a hatching factor*

	Mean number larvae per root system		
[1]Plant	[2]*H. schachtii*	[2]*H. rostochiensis*	[3]*H. göttingiana*
Beet	220	19	12
Pea	69	42	200
Tomato	3	147	7
Clover	6	5	11
Aster	1	77	—
Oats	38	34	—
Grass	2	—	—
Hesperis	135	—	—
Cabbage	—	24	—
Solanum nigrum	—	42	—

— Not tested.
[1] For host status, see Table 1.
[2] Mean of 4 replicates.
[3] Mean of 2 replicates.

The results in Table IV show that the presence of roots of a host does not affect the number of larvae that invade the roots of a non-

TABLE IV

Comparison of invasion by larvae of H. schachtii *in roots of a plant not producing a hatching factor for this eelworm, in the presence and absence of a host plant*

Pairs of plants grown together	[1]No. of larvae in root system	Total larvae invading per pot
Pea	230	506
Pea	276	
Sugar beet	611	1123
Sugar beet	512	
Pea	239	850
Sugar beet	611	

[1] Mean of 4 replicates.

host which does not produce a hatching factor. This suggests that the hatching factor is not necessarily concerned in attraction to and invasion of roots.

DISCUSSION

In terms of population build-up, quite different results may be obtained under field conditions from those in pot experiments. JONES (1956) found that, in microplots, cabbage built up the population level of beet eelworm more than sugar beet. Here, differences in plant numbers, habit and growth rate of the root system, length of life in the ground, root diffusate production and its effect on hatch, all play a part.

Variations can also occur within lines of the same species. GOLDEN & SHAFER (1958) report differential response to beet eelworm by selections of *Chenopodium album*. In a microplot experiment with several lines of sugar beet and *B. maritima*, all decreased the population from a moderately high level, except one line of *B. maritima*, which increased it (Table V). The difference between this line and the rest is statistically significant at the 0.1% probability level. The root system of this line was very extensive, a factor which may have been partly responsible for the difference.

TABLE V

Effect of various lines of sugar beet and B. maritima *on population levels of beet eelworm, in terms of eggs/g. air-dried soil, in microplots*

	Line	[1] Mean population level Initial	Final	Difference
Sugar beet	1	48	25	—23
Sugar beet	2	43	26	—17
Sugar beet	3	41	30	—11
B. maritima	1	46	31	—15
B. maritima	2	40	29	—11
B. maritima	3	43	31	—12
B. maritima	4	45	35	—10
B. maritima	5	42	47	*+5

[1] Mean of 8 replicates
* Significant at 0.1%.

Different populations of an eelworm species may also respond differently to the same plant species. JONES (1957), DUNNETT (1957), HUIJSMAN (1957), VAN DER LAAN & HUIJSMAN (1957) and GOFFART (1957) found populations of potato root eelworm which will form mature females on lines of wild potato, *Solanum tuberosum* subsp.

andigena, which did not support females in previously tested populations. Again, PRUMMEL (1958) found that two strains of potato root eelworm from different regions developed differentially on two lines of *Solanum nigrum* from the same source as the nematodes.

Little is known of the genetics of resistance to nematodes, either on the part of the host or parasite, or of the way in which the genetic differences are expressed in terms of the physiological interaction of host and parasite. The giant cells associated with the head of the feeding larva are obviously very important, but their significance is not clearly understood. KÜHN (1958) showed that necroses develop in the cells associated with the head of the invasive larva of *H. rostochiensis* in the roots of resistant *Solanum tuberosum* subsp. *andigena,* where giant cells would form in a susceptible potato plant. He states that the nematode relies for its nutrition on the giant cells formed as a reaction by the plant cells to the injection of saliva by the larva, and dies in their absence.

NUSBAUM (1958) found that maleic hydrazide applied to the leaves of tobacco plants infested with the root-knot nematode (*Meloidogyne incognita*) inhibited giant cell formation and prevented development of mature nematodes. However, when a lighter dose was given to infested and uninfested plants, the infested plants grew taller, suggesting that nematodes produced or caused the plant to produce a growth factor.

JONES (1954), in discussing resistance to potato root eelworm, suggests three arbitrary categories into which plant species can be placed, as follows:

1. Absolutely resistant. Not invaded.
2. Partially resistant. Invaded.
    a. Larvae fail to develop.
    b. Larvae develop but fail to mature.
    c. Larvae mature, but females are reduced in numbers and not very prolific.
3. Susceptible. Large numbers of highly prolific females produced.

The results of these investigations with *H. schachtii* show that the situation is even more complex. The scheme might perhaps be modified as follows:

1. Absolutely resistant. Not invaded.
2. Partially resistant. Invaded.
    a. Larvae fail to develop past the invasive stage.
    b. Some larvae develop to the third stage.

c. Some larvae develop into mature males, but females absent or very few in number.
d. Mature individuals of both sexes developed, but reduced in numbers and females not very prolific.

3. Susceptible. Large numbers of highly prolific females produced.

But even this still oversimplifies the real situation. The relationship appears to be continuous rather than discontinuous, with variations occurring not only from species to species, but from plant to plant within a species and, between the extremes of complete immunity and complete susceptibility, the whole range of host efficiencies exists. The first category of the scheme may not exist, although more work is needed to verify this. In the field, wherever there is a population of cysts in the soil, free larvae are always present at favourable seasons, either in small numbers from the limited spontaneous hatch which occurs in most species, or in greater numbers in the presence of a host crop. These experiments suggest that larvae may enter any roots with which they come into contact, even if host roots are also present, and although the majority will probably die shortly after entry, some may develop to maturity. The host-parasite relationship, therefore, is best expressed as a frequency. For example, when tested with an inoculum of Q larvae, P reached maturity and reproduced on variety X of plant Y. The ratio P/Q is the frequency in question. For marginal hosts in tests using small numbers of larvae, P/Q will usually be 0, but using large numbers, especially when drawn from several populations, P/Q may have a low but definite value. The ability of some individuals to develop on plants usually regarded as non hosts gives rise to some interesting questions and probably has a bearing on the origin of biotypes, biological races and speciation, not only in *Heterodera* but in other species of plant parasitic nematodes as well.

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ZUSAMMENFASSUNG

Eindringen und Entwicklung einiger Heterodera-*Arten in Wirtspflanzen verschiedenen Grades*

Das Wirts-Parasit-Verhältnis zwischen *Heterodera schachtii* und 13 Pflanzenarten aus den Familien der Chenopodiaceen und Cruciferen wurde untersucht. Die Pflanzen wurden mit sterilisiertem Kompost in 6 cm Töpfen angezogen und jede

mit 2000 Rübennematodenlarven beimpft. Eine Woche später wurden die Wurzeln vorsichtig von Boden gesäubert und die Pflanzen mit unverseuchtem Kompost in 10 cm Töpfe überführt. In wöchentlichen Abständen wurden 4 Pflanzen jeder Art 5 Wochen lang vom Tage der Beimpfung an gerechnet aufgenommen, die Wurzeln fixiert und in Formol-Eisessig-Alkohol aufbewahrt. Nach Färben mit Säurefuchsin und nach Mazerierung in einem Mixer erfolgte ihre Untersuchung auf Nematoden.

Larven III wurden in allen Pflanzenarten gefunden, wenn auch öfters nur in kleiner Zahl; Larven IV traten in *Beta webbiana, B. patellaris, B. procumbens, Hesperis* und *Matthiola* auf. Männchen fanden sich nicht in *B. procumbens, Hesperis* oder *Matthiola* und nur einmal in *B. webbiana*. Weibchen wurden in Wurzeln von *B. procumbens, B. patellaris, Hesperis* und *Matthiola* nicht gefunden; sehr wenige Weibchen traten in Radies, *Chenopodium album* und *Brassica sinapis* und nur einmal in *Beta webbiana* auf.

B. trigyna ist eine bessere Wirtspflanze als Zuckerrübe; anfällige Chenopodiaceen-Arten sind besser als anfällige Cruciferenarten. Die Zahl der Larven, die anscheinend schon tot waren, bevor die Wurzeln fixiert wurden, war hoch, selbst in anfälligen Arten; die meisten Toten scheinen im zweiten und dritten Larvenstadium aufzutreten.

Wenn geschlüpfte Larven von *H. schachtii, H. rostochiensis* und *H. göttingiana* Töpfen zugefügt wurden, in denen Pflanzen stehen, die keinen Schlüpfreiz produzieren, werden alle Arten befallen, wenn auch zuweilen nur von sehr wenigen Larven. Die Anwesenheit von Wirtspflanzenwurzeln wirkt nicht auf die Zahl der Larven von *H. schachtii,* die Erbsenwurzeln befallen, welche selbst keinen Schlüpfstoff für diesen Nematoden bilden.

Es werden die Faktoren diskutiert, die auf das Verhältnis Wirt-Parasit einwirken. Eine Veränderung der Jones'schen Klassifizierung von Pflanzen in Kategorien ihres Wirtspflanzengrades wird angegeben. Die Beziehung wird am besten als Frequenz ausgedrückt, z.B., wenn eine Impfung mit Q Larven erfolgt und P Larven die Reife erzielen und sich vermehren an der Sorte X der Pflanze Y, dann ist P/Q die Frequenz. Sie kann 0 sein, wenn Y eine Randwirtspflanze und Q klein ist; sie kann aber einen bestimmten Wert haben, wenn Q gross ist.

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HOPLOLAIMUS ABERRANS N.SP. (HOPLOLAIMINAE : TYLENCHIDA)

BY

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A single female specimen of a new species of the genus *Hoplolaimus* von Daday, 1905 was obtained from about the roots of nursery virginian tobacco (*Nicotiana tabacum* L.), variety Bonanza on a farm in Iringa district, Southern Highlands Province, Tanganyika Territory. As this species is so distinct from other *Hoplolaimus* spp., the unusual procedure of designating a new species from a single specimen has been adopted. If further specimens are obtained a redescription will be published, together with a full discussion of the taxonomic status of the species, and of the genus *Hoplolaimus*.

Hoplolaimus aberrans n.sp.

I ♀ L = 0.87 mm.; a = 23.5; b = 6.4; c = 49.7; V = 59.0%; Stylet = 31 μ.

Body — stout and only slightly curved.

Head — cap-like, but slightly offset and a little wider than body immediately behind constriction. Eight annules on the head. Skeletal framework of head very strongly developed, outer margins of basal plate extending into first annule behind head. The striking characteristic of the head is the complete absence of longitudinal striae on the annules.

Stylet strongly formed, basal knobs each bearing three teeth on anterior margin. Spear guiding apparatus indistinct, only part of a single ring observed.

Oesophagus resembles that of other *Hoplolaimus* spp. but neither nuclei of, nor outlets from dorsal and subventral gland cells to oesophageal lumen, could be traced. Intestine highly granular, rectum and anus well developed, the latter lying on thirteenth annule from tail tip.

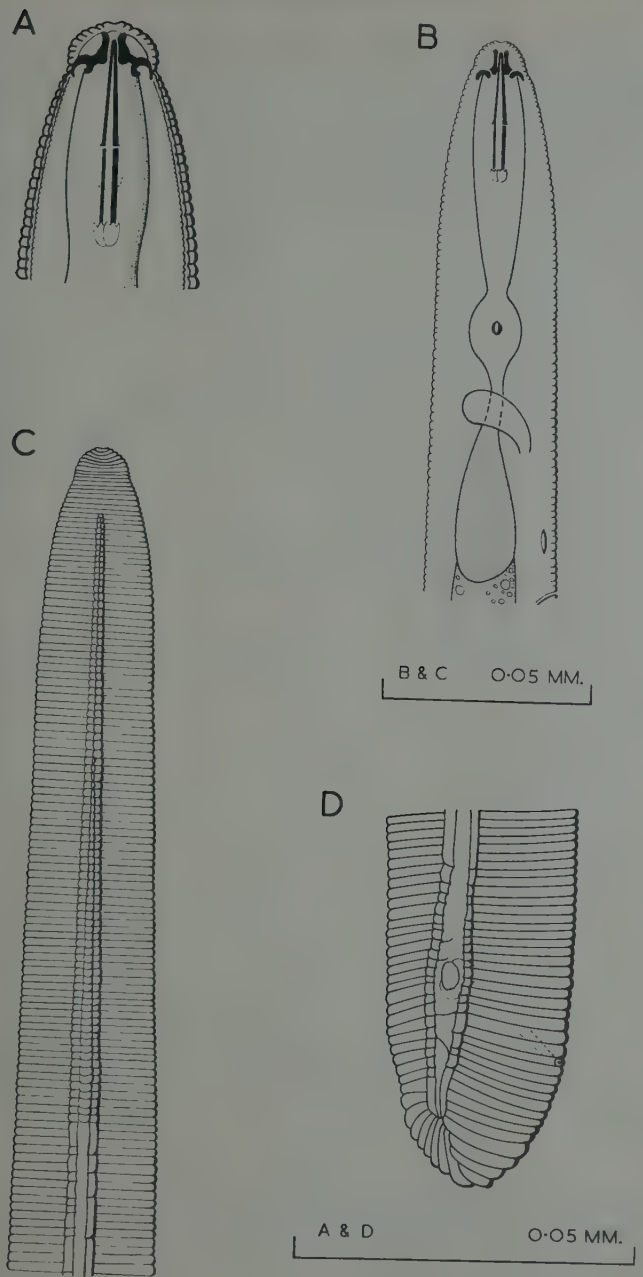

Figure 1. *Hoplolaimus aberrans* n.sp. ♀ ; A. Head end; B. Oesophageal region; C. Anterior body cuticle; D. Tail.

Paired ovaries with oocytes in single row throughout. No spermathecae, probably indicating absence or rarity of males.

Lateral field of cuticle arising some eight annules behind head and consisting of two bands between this point and centre of median bulb of oesophagus, where middle incisure bifurcates. Transverse striation of middle band of lateral field beginning at about middle of isthmus and continuing to a point but seven annules behind metacorpus; posteriorly the two outer bands of field irregularly striated. One tail's length in front of scutellum (phasmid) cross-striation becomes more regular until just behind level of anus, posterior to which outer bands smooth. Only two complete striae occur on central band of field behind scutellum. In front of scutellum two incomplete striae observed. Absence of striation on greater part of central band of lateral field is another outstanding feature of this species. Annules on middle of body approximately 1.5 μ wide.

Excretory pore situated at level of posterior margin of metacorpus but hemizonid lies five annules in front of pore.

Scutella (phasmids) located in vicinity of anus unlike all other species of genus are opposite.

Holotype: a single female specimen with above measurements, on slide N.t. 43, in slide collection of Nematology Department, East African Agriculture and Forestry Research Organisation, P.O. Box 21, Kikuyu, Kenya.

Type locality: probably ectoparasitic on the roots of nursery virginian type tobacco (*Nicotiana tabacum* L.) of the variety Bonanza, found on Souvaris' Farm, Iheme, Iringa District, Southern Highlands Province, Tanganyika Territory.

Diagnosis: *Hoplolaimus* with above characters. Head cap-like, slightly offset, with eight annules but no trace of longitudinal striation; stylet knobs each bear three teeth; hemizonid located some five annules in front of excretory pore; lateral field with four incisures, middle band being striated between isthmus and a point slightly behind proximal extremity of metacorpus; central band of lateral field only slightly striated in region of scutellum; outer bands of lateral field irregularly striated over most of their length; phasmids (scutella) large, opposite and adanal.

RESUMÉ

L'auteur donne une description de *Hoplolaimus aberrans* n.sp. La nouvelle espèce se distingue si nettement des autres espèces du genre, que l'auteur s'est basé sur un seul exemplaire, une femelle, pour la décrire.

Footnote: The author has recently made a morphological study of adults of *Scutellonema bradys* (Steiner & Le Hew, 1933) Andrássy, 1958 upon which the redescription of this species by T. Goodey (1935) was based. Certain features of the head and tail regions of the female were inadequately described by this author and *H. aberrans* is fairly close to this species. This will be further discussed in a subsequent paper when more specimens of *H. aberrans* are obtained.

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THE ROOT-KNOT NEMATODES OF EAST AFRICA

1. *MELOIDOGYNE AFRICANA* N. SP., A PARASITE OF ARABICA COFFEE (*COFFEA ARABICA* L.)

BY

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Root-knot nematodes (species of *Meloidogyne* Goeldi, 1887 (emend. Allen, 1952)) frequently attack arabica coffee in the mountain areas of East Africa. Coffee nurseries are widely attacked in the Meru District of Kenya by a new species *Meloidogyne africana* n.sp. A species with a very similar anal plate pattern in the female also attacks arabica coffee on Mt. Kilimanjaro, Tanganyika. Galls due to the invasion of the roots by this species are usually small, from 1 to 5 mm in diameter. Affected seedlings are generally stunted and chlorotic, while feeder roots and often main roots also, are 'blinded', resulting in the formation of numerous branch rootlets behind the affected root-tip. Heavy attacks in mature coffee trees by *Meloidogyne* spp. are generally associated with unthriftiness.

Meloidogyne africana n. sp.

(Figs. 1-2)

MEASUREMENTS

17 females: L = 0.66-0.91 mm ($\bar{x}$ = 0.76 ± 0.02); a = 1.4-1.9 ($\bar{x}$ = 1.59 ± 0.04); Spear = 15 μ.

18 males: L = 1.20-1.85 mm ($\bar{x}$ = 1.47 ± 0.05); a = 31-50 ($\bar{x}$ = 38.9 ± 1.1); b = 3.8-6.7 ($\bar{x}$ = 5.1 ± 0.3); c = 69-103 ($\bar{x}$ = 86 ± 2); Spear = 19-22 μ. ($\bar{x}$ = 20.7 ± 0.3); Spicules = 26-35 μ; Gubernaculum = 7-9 μ.

Holotype male: L = 1.32 mm; a = 39; b = 5.1; c = 88; Spear = 21 μ.

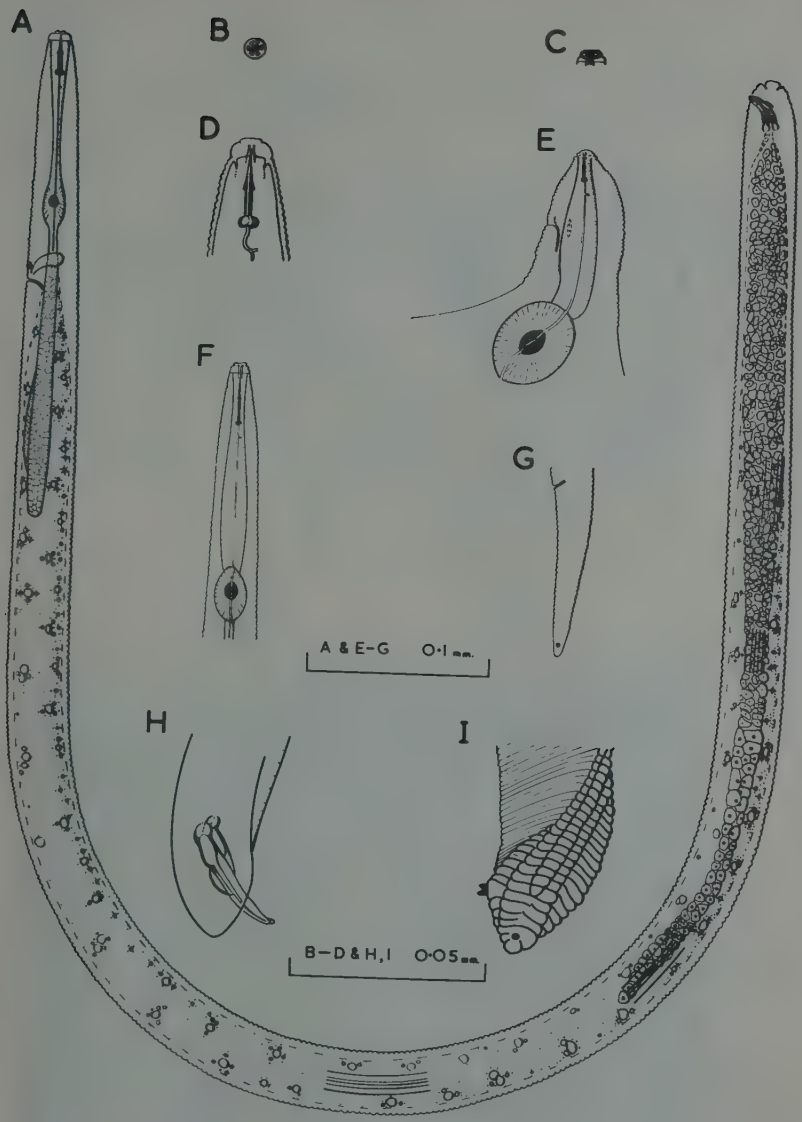

Fig. 1A. Male *M. africana;* B *En face* view of head of female; C Lateral view of head of female; D Anterior end of male; E Anterior end of female; F Anterior end of larva; G Tail of larva; H Tail region of male to show copulatory apparatus; I Tail region of male to show cuticular pattern.

25 Infective larvae[1]): L. = 0.38-0.47 mm ($\bar{x}$ = 0.42 ± 0.005); a = 22-28 ($\bar{x}$ = 24.4 ± 0.4); b_1[2]) = 6.1-7.8 ($\bar{x}$ = 7.1 ± 0.1); c = 7.3-14.3 ($\bar{x}$ = 10.8 ± 0.5); Spear = 12-18 μ ($\bar{x}$ = 14.8 ± 0.3).
25 Eggs: 81-102 μ ($\bar{x}$ = 91.6 ± 1.0) X 44-48 μ ($\bar{x}$ = 45.3 ± 0.3).

DESCRIPTION

Female: Body pyriform, anterior conical, posterior end broad; perianal region somewhat elevated. Head truncated cone, bearing terminal labial region made up of six equal lips, succeeded by a single annule. Head skeleton of very thin basal plate with guide ring extending backwards into first body annule and enveloping anterior end of spear. Stylet small, basal knobs rounded, or tending to flatten anteriorly. Dorsal oesophageal gland duct aperture lying 4-9 μ behind spear base. Median bulb of oesophagus large, ovate, with large crescentic valves. Excretory pore 16-30 annules behind the head. Hemizonid apparent in one specimen (Fig. 1E). Cuticular pattern of the perianal region

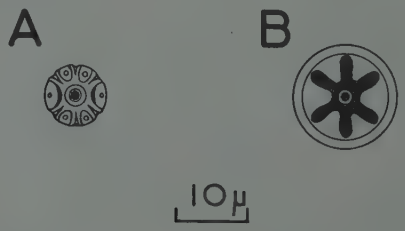

Fig. 2 A. En face view of male *M. africana*; B. Transverse optical section of male *M. africana* at basal plate level.

characteristic, composed of smooth striae forming a "low" dorsal arch; tail terminus wide, generally covered with short, coarse, disordered striae and surrounded by concentric circles of striae forming a distinct raised tail pattern; phasmids adjacent to tail terminus; lateral field extending from each phasmid along side of body as wide, irregular area, without incisures and coarse striae, but very short, faint striae

1) The largest preparasitic stage found from both soil and root suspensions.
2) Owing to the difficulty of tracing the extent of the posterior oesophageal region of the larva, the ratio of the length of the body anterior to the mid-point of the valves of the median bulb, to the total body length (i.e. b_1) is taken instead of the accepted convention b, which is the ratio of the body length anterior to the posterior end of the oesophagus, to the total body length. It is suggested that b_1 might profitably be used in taxonomic studies of genera with obscure posterior glandular regions.

A. G. WHITEHEAD: *Meloidogyne africana* n.sp.

Fig. 4. Tracing of Fig. 3 to emphasise morphological characteristics.

Fig. 3. Photograph of perianal cuticular pattern of female *M. africana*.

may be seen in each field. Lateral fields do not extend far beyond edge of pattern illustrated (Fig. 4).

Male: Body cuticle finely striated; lateral fields occupy about ¼ body width, exhibiting five incisures mid-body. At the level of oesophageal gland four incisures only occur and the fifth incisure is lost towards posterior end of testis. Middle incisure frequently broken, the ends sometimes overlapping to form a sixth incisure for short lengths of the body. Lateral fields areolated on the tail-end. Phasmids close to tail-tip, equal and opposite.

En face view of head (Fig. 2 A) shows lateral lips larger than subdorsals and subventrals. Subdorsal and subventral lips each bear a large papilla, lateral lips each bear a smaller papilla. Amphidial apertures slit-like, situated close to oral cavity. Basal plate simple, thin and hexaradiate structure (Fig. 2B). Stylet fairly strong with large, rounded basal knobs, sometimes bearing fine anterior teeth. Aperture of dorsal oesophageal gland duct 4-6 μ behing spear base. Hemizonid 59-75 annules behind head; excretory pore either immediately behind hemizonid or 1-4 annules intervening. Median bulb of oesophagus ovate, with strong crescentic valves; glandular lobe long and narrow, often obscured by intestine but usually on right side of body. Testis single, rarely reflexed. Vas deferens wide, full of spermatocytes and spermatozoa, passing as ejaculatory duct between heads of spicules. Tail-end without bursa; lateral fields on tail-end areolated and marked by four incisures, of which the inner coalesce at cloacal level. Phasmids located close to tail-tip. Spicules equal, composed of head, open proximally, short shaft and long blade. Gubernaculum a thin, simple plate tapering towards its extremities, forming a shallow trough.

Larvae: Body fusiform; head composed of labial region and a basal segment, displaying two annules in lateral aspect but one only in dorsal or ventral view. Spear prominent with rounded basal knobs. Dorsal oesophageal gland aperture about 3 μ behind spear base. Median bulb of oesophagus prominent, ovate, with strong crescentic valves. Genital primordium located beneath intestine 53%-62% of the body length from the anterior margin of the head. Tail 30-48 μ long ($\bar{x} = 40 \pm 1.7$), tapering to a rounded terminus. Phasmids very close to tail-tip. No lateral field observed.

DIAGNOSIS

Meloidogyne. With above characters and measurements. Head consisting of labial region followed by one annule in adults; perianal region

of female with low dorsal arch composed of closely spaced smooth striae forming a distinct elevated whorl around the broad tail end; lateral fields wide, unmarked by incisures but containing very faint, short striae; lateral field of male marked by five incisures mid-body, four incisures on post-gonadial region, areolated on tail end. Phasmids of female, male and larva very close to tail end, equal and opposite.

Type specimens: — Holotype male No. 5 slide S. A. x.; Paratypes males Nos. 1-4 and 7-19 slide C. A. x., Nematology Department, E.A.A.F.R.O., Box 21, Kikuyu, Kenya.

Type host: — Roots of *Coffea arabica* L.

Type locality: — Kamaara Coffee Nursery, Meru District, Kenya, Africa.

DISCUSSION

M. africana is the ninth species of the genus *Meloidogyne* to be described. It differs from the other eight species, two subspecies and one variety in the form of the perineal cuticular pattern of the female, in the presence of five incisures on the lateral field on the mid-body of males, in the areolation of the lateral field on the male tail and in the very posterior position of the phasmids in all stages of the species.

Like *M. acronea* Coetzee, 1956, *M. africana* has post-anal phasmids but the female of *M. acronea* is said to have no distinct perianal pattern. In the possession of but one post-labial annule *M. africana* resembles *M. inornata* Lordello, 1956.

Coffea arabica L. is known to be attacked by two species of *Meloidogyne*, viz., *M. exigua* Goeldi, 1887, which is also the type species of the genus, and *M. africana*. A comparison of the morphology of these two species is necessary.

M. exigua occurs in South America, especially in Brazil and has been recently redescribed (Lordello & Zamith, 1958). *M. africana* is at present only known in East Africa and *M. exigua* is unknown in East African coffee. *M. africana* resembles *M. exigua* in that the male head of both species has only one post-labial annule and in so far as there may be more than four incisures on parts of the lateral field. However, the males of *M. africana* unlike those of *M. exigua* have a twisted body, the amphids are not prominent in lateral view of the head, the outer bands of the lateral fields are not cross-striated, except in the vicinity of, and on, the tail, and there is but one testis. The male body of *M. africana* is longer (1.20-1.85 mm) than that of *M. exigua* (0.83-1.09 mm). The perianal cuticular pattern of *M. africana* has a

distinct tail whorl with the phasmids close to the tail terminus, while the lateral fields are wide and contain very short, faint, disordered striae. The perianal pattern of *M. exigua,* however, has no distinct tail whorl and the lateral fields are poorly defined and do not contain fine, short striae. Unfortunately the *en face* views of the heads of adult specimens of *M. exigua* are not illustrated either by Chitwood, 1949 or by Lordello & Zamith, 1958 so it is not possible to make a comparison of labial papillae. Indeed the only species for which *en face* views are available are *M. hapla* Chitwood, 1949, *M. incognita* var. *acrita* Chitwood, 1949 and *M. brevicauda* Loos, 1953. In none of these species are labial papillae shown and Allen (1952) makes no mention of them in his emendation of the generic diagnosis. It seems probable that large papillae are not found in species other than *M. africana,* though in this species they are only found in the male.

The perianal pattern of the cuticle of the adult female partly resembles that of a pre-adult female of *M. javanica* (Treub, 1885) Chitwood, 1949, and may add support to the author's belief that *M. africana* may lie close to the ancestry of the genus. Further investigation of this matter is being pursued.

RESUMÉ

L'auteur décrit *Meloidogyne africana* n.sp. *M. africana* diffère des autres espèces de ce genre par la forme circulaire caractéristique de la partie anale chez la femelle, avec un arc dorsal "bas", de larges côtés latéraux non marqués de raires longitudinales, mais présentant des stries légères et très courtes; le bout de la queue est large et applati et couvert de stries irrégulieres et rares. Les mâles, qui sont nombreux, ont des côtés latéraux marqués de cinq raies longitudinales sur la plus grande longueur du corps, mais seulement quatre sur le bout de la queue où elles forment un quadrillage. Les phasmides des femelles, des mâles, et des larves se trouvent à proximité du bout de la queue.

On sait que *M. africana* attaque les jeunes caféiers arabiques (*Coffea arabica* L.), il en resulte une croissance sclérosée, des racines courtes et tordues. Les galles des racines mesurent de là 5 mm de diamètre.

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TRICHOTYLENCHUS FALCIFORMIS N.G., N.SP., (BELONOLAIMINAE N. SUBFAM.: TYLENCHIDA THORNE, 1949) AN ASSOCIATE OF GRASS ROOTS (*HYPARRHENIA* SP.) IN SOUTHERN TANGANYIKA

BY

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Examination of nematodes obtained by the Baermann Funnel technique from a mixture of the roots and surrounding soil of a grass (*Hyparrhenia* sp.) collected at mile 40 on the Songea to Tunduru road, Southern Province, Tanganyika Territory, disclosed a few adults and several larvae of a new nematode genus and species — *Trichotylenchus falciformis* n.g., n.sp.

Although *T. falciformis* shows striking differences from other members of the family Tylenchidae Filipjev, 1934, it shares with *Belonolaimus* Steiner, 1949 a number of important characters. It is therefore proposed that a new subfamily Belonolaiminae be erected to receive both genera, which as will be shown, are not readily accomodated in any of the existing subfamilies. *Belonolaimus* is preferred as type genus of the new subfamily not only on account of its earlier description but because it is more widely known (Rau, 1958).

Belonolaiminae n. subfam.

Diagnosis: *Tylenchidae* Filipjev, 1934 (sensu Thorne, 1949). Body long in relation to its width; tail more than twice as long as anal body width. Body cuticle striated, lateral fields marked by less than four incisures. Phasmids postanal, dereids not observed. Head with four or six lips, annulated. Spear long in relation to its breadth. Median bulb of oesophagus spherical or ovate, metacorpus long, overlapping anterior end of intestine; dorsal oesophageal gland duct opening into lumen of precorpus close to spear-base. Ovaries paired, vulva mid-body. Single testis in male, bursa extending to tail terminus, spicules tylenchoid.

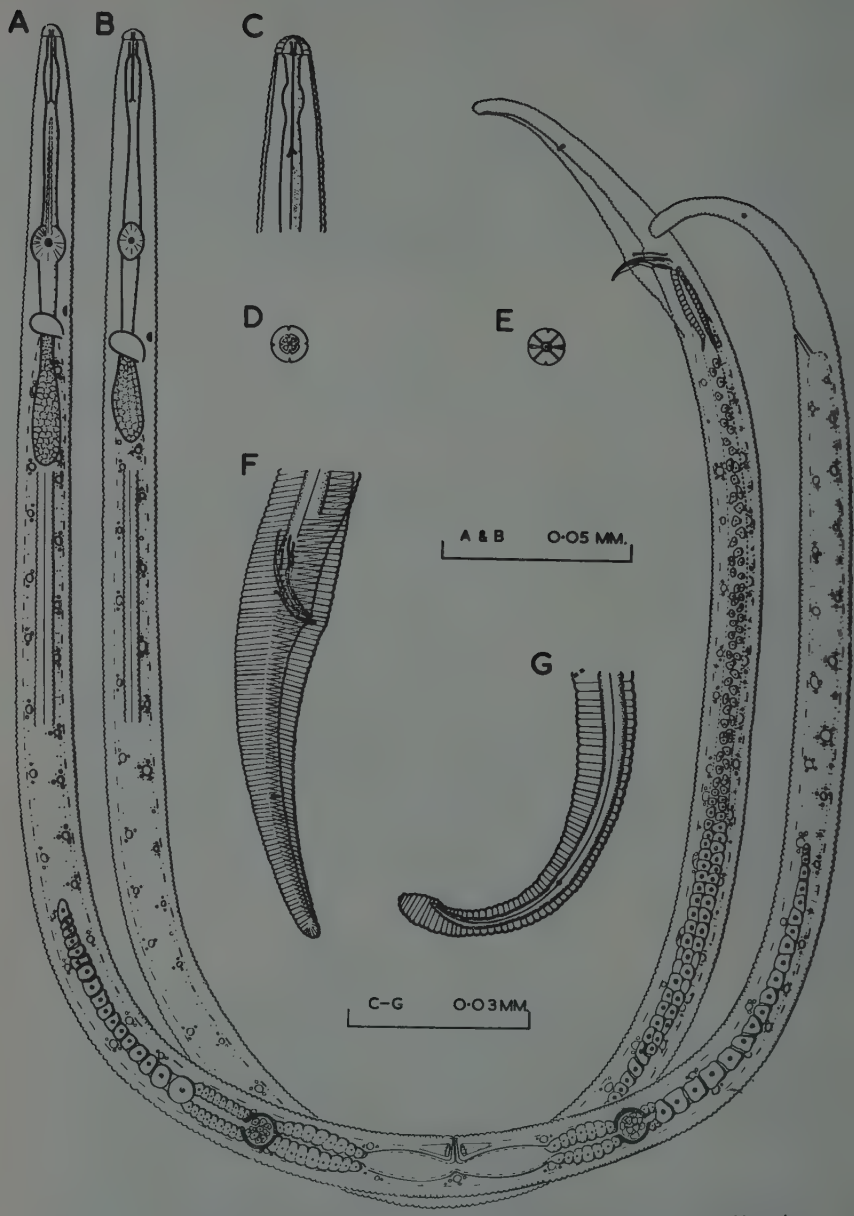

Fig. 1. A. Female *Trichotylenchus falciformis* n.g., n.sp.; B. Male *T. falciformis*; C. Female — Anterior end of body; D. Male — Head *en face* view of lip region; E. Male — Head *en face* view of basal plate; F. Male — Tail; G. Female — Tail.

Genera: — *Belonolaimus* Steiner, 1949 and *Trichotylenchus* n.g. *Belonolaimus* is here designated as type genus.

Trichotylenchus n.g.

Diagnosis: Belonolaiminae. Spear base forked, each arm bearing a rounded, basal knob. Tail long, tapering to a rounded terminus. Lateral field with three incisures. Head with four lips, marked by four or more annules.

Type species: *Trichotylenchus falciformis* n.sp.

(Fig. 1)

Measurements.

Females (9) L = 0.67-0.91 mm; a = 36-46; b = 4.4-7.1; c = 10.7-12.5; V = 49.4-60.0%
Spear length = 17-25 μ; Ratio ..... tail-length to anal-body-width = 5.0-7.1.

Males (3) L = 0.69-0.74 mm; a = 47-50; b = 5.6-6.5; c = 12.2-12.4.
Spicules = 15-18 μ. Gubernaculum = 8-10 μ.
Spear = 18-20 μ. Ratio ..... tail-length to anal body width = 5.3-6.6.

Holotype female L = 0.67 mm; a = 42; b = 5.2; V = 49.4%

Allotype male L = 0.74 mm; a = 47; b = 6.5; c = 12.2

Description. — Body narrow, sickle-shaped (hence trivial name); cuticle finely striated, striations of subcuticle twice as numerous as those on most of the cuticle but some areas of the body carry cuticular striations each of which exhibits a secondary stria. Tail tapering to bluntly rounded, striated terminus, striae following contour of the tail terminus. Lateral fields prominent, originating some three or four annules behind the spear-base; marked by two incisures to about nine annules behind spear-base, where the field is divided into two bands by a median incisure. Outer incisures of lateral field crenate. Phasmids small, rounded, interrupting the median incisure at 40-57% of the tail length in females and 53-64% in males. The phasmids of the male, however, extend into the bursal wings as a pair of 'ribs'. The lateral field ceases close to tail terminus in female and in vicinity of anus in male.

Head contour continuous with body contour, bearing four or more very faintly marked annules; head divided into four lobes with four lips in *en face* view. Head framework thin, basal plate composed of four main arms and four minor lateral arms (Fig. 1E).

Wall of buccal region with a characteristic swelling at level of forepart of spear. Spear needle-like, spear-base bifurcate, both arms bearing a rounded basal knob. Dorsal oesophageal gland duct opening into oesophageal lumen about 2 μ behind spear base. Median oesophageal bulb with small crescentic valves. Oesophago-intestinal junction immediately behind median bulb. Metacorpus a long, narrow, glandular lobe, overlying right or left side of intestine. Nuclei not observed in this glandular region nor the opening of the ventral oesophageal gland duct.

Hemizonid two annules long, situated 55-70 annules behind head and lying half-way between median bulb and end of oesophagus on ventral body surface. Excretory pore not seen.

Intestine granular, rectum and anus apparently functional.

Female. Two outstretched ovaries with oogonia in a single line. Spermatheca present at junction of ovary with uterus. Each uterus composed of thick-walled distal half and thin-walled proximal half, leading to vagina. Vulva situated halfway along ventral body surface.

Male. Single testis with spermatogonia in single or double line. Vas deferens narrow, containing relatively few spermatozoa. Spicules of usual tylenchoid shape. Gubernaculum thin, proximal end curved away slightly from spicules; a 'titilla' of very small size on each side of the distal end. Bursal wings arising some distance in front of spicule heads, crenate, and extending to tail terminus.

Type Specimens. Holotype female No. 5 slide Grass 88/A, Allotype male No. 4 slide Grass 88/B, in collection of Nematology Department, East African Agriculture and Forestry Research Organisation, P.O. Box 21, Kikuyu, Kenya.

Type Locality. Around the roots of a grass — *Hyparrhenia* sp., mile 40 on the Songea to Tunduru road, Southern Province, Tanganyika Territory.

DISCUSSION

Trichotylenchus is closely related to *Belonolaimus*. In both genera the body is narrow in relation to its length, the ovaries are paired, opposed and outstretched, the vulva lies mid-body, the spear is slender, the phasmids are small and postanal, the posterior oesophageal region

overlaps the intestine and the bursa envelops the tail terminus in the male. However, *Trichotylenchus* has a bifurcate spear-base, four lips, a tapering tail and the lateral field bears three incisures, while in *Belonolaimus* the spear base is unforked, there are six lips (RAU, 1958), the tail is cylindrical and the lateral field is marked by a single incisure. The two genera are therefore distinct, but show similarities justifying their inclusion in the same subfamily.

The taxonomic postion of *Belonolaimus* has been problematical since its original description by STEINER (1949). CHITWOOD (1950) placed *Dolichodorus* Cobb, 1914 and *Belonolaimus* in the subfamily Dolichodorinae of the family Criconematidae Thorne, 1943 (defined Thorne, 1949) but as shown by LOOF (1958) these two genera are only superficially related. LOOF concludes that *Belonolaimus* keys out to Hoplolaiminae Filipjev, 1934 (emend. Thorne, 1949). While *Dolichodorus* probably belongs to the Criconematidae, *Belonolaimus* and *Trichotylenchus* best fit the family Tylenchidae Filipjev, 1934.

Tylenchidae contains at present four subfamilies — Tylenchinae Filipjev, 1934 (emend. Thorne, 1949), Hoplolaiminae Filipjev, 1934 (emend. Thorne, 1949), Pratylenchinae Thorne, 1949 and Rotylenchoidinae Whitehead, 1958. Belonolaiminae following THORNE's classification of the order Tylenchida (1949) would therefore constitute the fifth subfamily. Unlike Tylenchinae, Belonolaiminae do not have a distinct terminal oesophageal bulb. Unlike Hoplolaiminae, Belonolaiminae do not have a heavy head skeleton, the tails of both sexes are very much longer than the anal body width, the phasmids are post-anal, which is rare in Hoplolaiminae, the tail may or may not taper, those of Hoplolaiminae being generally cylindrical and there are less than four incisures in the lateral field. Unlike Rotylenchoidinae, Belonolaiminae have two ovaries and the vulva is medial, not subterminal. Pratylenchinae is such a loosely associated group of genera in THORNE's system that its member genera share hardly any important characters. Therefore apart from the much greater length of the spear and unflattened head of the new subfamily it is not easy to distinguish Belonolaiminae from this 'rest group'. Inclusion of the genera *Belonolaimus* and *Trichotylenchus* in Pratylenchinae is undesirable as there would thereby cease to be any useful diagnosis of the subfamily. Similarly their introduction to the subfamily Hoplolaiminae would only destroy the homogeneity of this otherwise compact group. Separation of these two genera to a subfamily of their own would seem to be the simplest course which can be followed, without undue disruption of existing groups.

Certain anatomical features of *Trichotylenchus* are unusual and justify further comment. Thus the forking of the spear base is a character shared, among Tylenchida, only with *Chitinotylenchus* (Micoletzky, 1922) Filipjev, 1934, belonging to Tylenchinae and provides an example of the parallel evolution of the same character in two distinct subfamilies. It is interesting that in *Trichotylenchus* the spear base possessed only *two branches* and not as would be expected three. The significance of the bulb on the buccal wall is unknown. Again the presence of three incisures in the lateral field is unusual among Tylenchida. Finally the apparent absence of lateral lips and recognisable amphid apertures is strange but neither were found on the head. Indeed *Trichotylenchus* probably merely represents the final stage in the suppression of the lateral lips which are already much reduced in *B. longicaudatus* Rau, 1958, a species very close to, if not synonymous with *B. gracilis* Steiner, 1949, from which it is said to differ on the basis of certain measurements including the length of the tail. However, Rau illustrates a tail of *B. longicaudatus* (Fig. 1E), which closely approaches the general proportions of the tail of *B. gracilis* Steiner. In would seem significant that although collections were made in many parts of Florida, U.S.A., including the type locality, *B. gracilis* was not encountered.

The author is grateful to Dr. J. Basil Goodey for valued criticism of the text and to Miss D. M. Napper, East African Herbarium, Nairobi for identification of the grass from around whose roots *Trichotylenchus falciformis* was obtained.

RESUMÉ

L'auteur décrit *Trichotylenchus falciformis* n.g., n.sp. (Trichotylenchinae n. subfam.: Tylenchidae). Les Trichotylenchinae comportent à présent deux genres, *Belonolaimus* Steiner, 1949 et le genre-espèce *Trichotylenchus*. Les Trichotylenchinae diffèrent des autres sous-familles par la possession de très fines pointes comme des aiguilles, moins de quatre raies longitudinales sur le côté latéral, la queue deux fois plus longue que la largeur du corps à la hauteur de l'anus, la tête divisée en quatre lobes et avec quatre lèvres, les glandes oésophagiennes postérieures recouvrant l'intestin, la présence de deux ovaires, phasmides postanaux chez la femelle, et un lobe enveloppant le bout de la queue chez le mâle. *Trichotylenchus* diffère de *Belonolaimus* par une queue effilée plus longue chez les deux sexes, la base bifurquée pointue, et la présence de trois raies longitudinales sur le côté latéral.

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NACOBBUS SERENDIPITICUS N.SP., A ROOT-GALLING NEMATODE FROM TOMATOES IN ENGLAND

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In September 1957 galled roots of glasshouse tomatoes were received from a nursery in Berkshire (Fig. 1). The galls were small and the many lateral roots associated with them suggested an infestation of *Meloidogyne hapla*. When the galls were dissected, however, a species of *Nacobbus* (Nacobbinae: Hoplolaimidae: Tylenchida) was found. Each gall contained two or three mature females, usually with egg masses in which there were sometimes one or two males. Certain morphological details distinguish these nematodes from the three known species of *Nacobbus* and they are here considered as a new species, which is given the name *N. serendipiticus* n.sp., recording the fact that their discovery was unexpected, because the three known species of the genus all occur in arid regions in the central and western United States of America. ('Serendipity' is the faculty of making happy and unexpected discoveries by accident).

In the following descriptions the measurements of males, females and eggs are from specimens extracted from roots which had been plunged into boiling cotton blue lactophenol and mounted in the same medium.

Nacobbus serendipiticus n.sp.

Females: Figs. 2a, b; 3a, b.

Length 0.9-1.6 mm; breadth: 0.26-0.42 mm [n = 10]; stylet: 19-22 μ (20.3 μ) [n = 12].
Holotype: L = 1 mm; B = 0.16 mm; a = 6.4; b = 11.8; c = 45.6; V = 94%; stylet = 20 μ.
Slide No. 59/4/1 in Rothamsted Nematology Collection.

The mature females are irregularly spindle-shaped with narrow neck region and subterminal vulva and anus. Annules are visible only on the

M. T. FRANKLIN: *Nacobbus serendipiticus* n.sp.

Fig. 1. Tomato seedling showing root galls due to *Nacobbus serendipiticus* n.sp.

narrow anterior and posterior regions. The specimen chosen as holotype is a young mature female because in older specimens the internal structures are much obscured by the coiled ovary and the eggs. The neck region narrows rapidly to a small head with three annules and a labial disc. The anus is close to the tip of the tail, the vulva 1-2 times the tail length in front of the anus and the phasmids slightly post-anal. A well-developed hexaradiate head-skeleton forms the stylet guide; the stylet is tylenchoid with rounded knobs (Fig. 3a). The anterior region of the oesophagus (procorpus) is well-developed, at its widest part more than half the width of the muscular bulb, but constricted at its junction with the bulb. The latter occupies about 3/5 the body width at this point and has conspicuous central plates. The glandular region of the oesophagus overlaps the intestine dorsally for 3-4 bulb lengths; the nuclei are not clear but the small posterior gland can sometimes be distinguished. Behind the oesophageal bulb a short isthmus, encircled by the nerve ring, quickly widens into the much swollen intestine. The dorsal oesophageal gland duct joins the lumen of the oesophagus about 2 μ behind the stylet base. The excretory pore is 1-2 bulb lengths behind the oesophageal bulb. The single ovary is very much coiled and may extend as far forward as the oesophageal bulb. Anteriorly the ovary has a single cell followed by a region of cell multiplication until there are about a dozen in cross-section. Behind this the numbers decrease until a single row of oocytes leads to the uterus, the thick walls of which are formed from one layer of cells. In dissections of nine or ten specimens and examinations of whole mounts of many others, from young females to mature forms containing eggs, no swollen spermatheca was seen such as has been described for *N. batatiformis* by THORNE & SCHUSTER (1956). In the young unswollen female the vulva-anus distance is about 1½ times the tail length and the phasmids are only slightly behind the anus, as in the mature female (Fig. 3b).

Males: Figs. 2e, f; 3c, d.

Length: 728-930 μ (830 μ); breadth: 25-30 μ (29 μ); a = 23-32 (29) [all n = 9]; b = 6-13 (10) [n = 10]; c = 31-53 (40) [n = 8]; stylet: 22-27 μ (25 μ) [n = 12]; spicules: 24-29 μ (27 μ) [n = 10].

Allotype: L = 875 μ; B = 29 μ; a = 30; b = 11; c = 39; stylet: 26 μ; spicules: 27 μ.

Slide No. 59/4/2 in Rothamsted Nematology Collection.

The body is annulated, cylindrical, tapering slightly to the head,

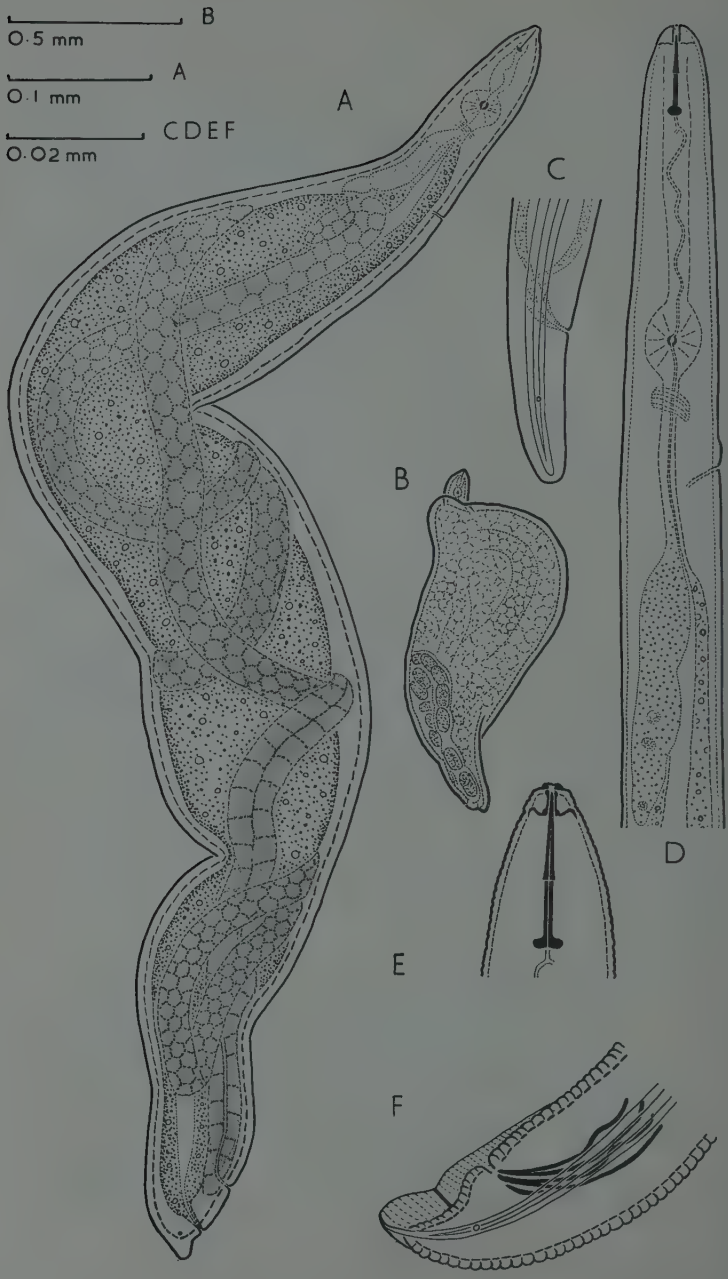

Fig. 2. *Nacobbus serendipiticus* n.sp. A. Young female. B. Mature female. C. Tail of larva. D. Oesophageal region of newly hatched larva. E. Male head. F. Male tail.

which is barely offset, and rapidly post-anally to the bluntly pointed tail. The head probably has four annules and a labial disc, but in some specimens only three annules could be distinguished. A strongly developed hexaradiate skeletal framework forms the stylet guide (Fig. 3c). The stylet is tylenchoid with prominent basal knobs, slightly cupped anteriorly (Fig. 2e). The lateral field has four incisures; it starts close

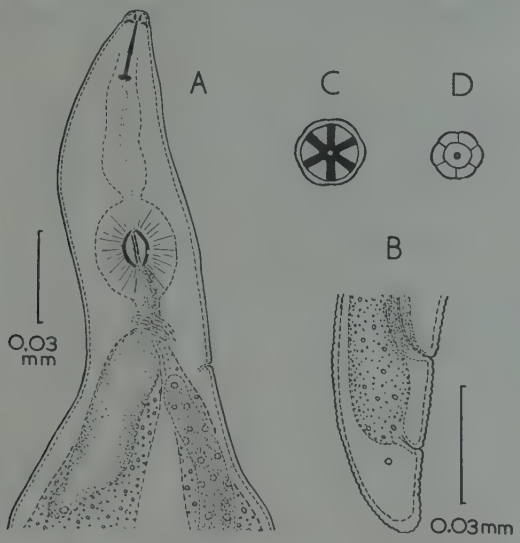

Fig. 3. *Nacobbus serendipiticus* n.sp. A. Oesophageal region of mature female. B. Posterior region of immature female. C. Optical section through male head showing hexaradiate skeletal structure. D. Surface view of male head.

behind the stylet base as a narrow band bounded on either side by an incisure, then expands for the greater part of the body length until the bursa is reached, where it is narrowed and the central incisures disappear (Fig. 2f); no deirid was seen. The excretory pore lies two to 2½ bulb lengths behind the oesophageal bulb, immediately behind the hemizonid. The oesophageal bulb is slightly more than half as wide as the body at the same point; it is followed by a narrow isthmus which widens into the intestine at about the region of the excretory pore. The glandular region of the oesophagus lies in a lobe dorsally over the intestine for a distance equal to about 2½ body widths.

The bursa arises close behind the level of the spicule heads and envelopes the tail; the phasmids form ribs on the bursa midway along

the tail. The spicules are tylenchoid and the gubernaculum simple, about one quarter the length of the spicules. The tail is about equal in length to the anal body diameter (Fig. 2f).

Larvae: Figs. 2c, d.

Length: 311-343 μ (328 μ); breadth: 12-15 μ (14 μ); a = 21-26 (23); b = 6; c = 13-14 (13.4) [*n* = 10 except for b where it is 5]; stylet: 13 μ [*n* = 3].

Larvae hatched from eggs kept in water were killed by gentle heat, fixed in 5% formalin, transferred through lactophenol to glycerine and measured after mounting. The body is cylindrical with bluntly rounded tail 2.6 times the anal body width. The annules are about 1 μ wide and the lateral field has four incisures occupying 2/5 of the body width. The phasmids lie midway along the tail. The lens-like hemizonid is in the usual ventral position one bulb-length behind the oesophageal bulb. The excretory pore and duct lie immediately behind the hemizonid but are difficult to see, especially in lactophenol-mounted specimens. The head is slightly offset, with three annules. The muscular bulb of the oesophagus is well-developed with conspicuous central plates; a narrow region about two bulb-lengths long and encircled by the nerve ring joins the intestine behind the excretory pore (Fig. 2d). The glandular lobe reaches to one third of the body length.

Slides Nos. 59/4/3 & 4. Rothamsted Nematology Collection.

Eggs: Length: 75-87.5 μ (80.4 μ); breadth: 35-41 μ (37.7 μ) [*n* = 15].

Eggs start to segment before they are laid but embryonated eggs were not observed within the females. No moult was seen in the eggs and the embryos have rounded tails. There does not appear to be a first stage larva with sharply pointed tail as described by Thorne & Allen (1944) for *N. dorsalis*.

Slide No. 59/4/5. Rothamsted Nematology Collection.

Relationships

The nematodes clearly belong to the genus *Nacobbus* because they show great sexual dimorphism and the male has tylenchoid spicules and a short pointed tail with a bursa surrounding the tip and the phasmids appearing as ribs on it. There is a head cap in both sexes. The oesophageal glands lie dorsally over the intestine. The female has a single coiled ovary and sub-terminal vulva and anus. The type species

of the genus, *N. dorsalis* Thorne & Allen, 1944, was found in galls on the roots of *Erodium cicutarium* L. growing in a sand pit in Kern County, California. *N. aberrans* (Thorne, 1935) Thorne & Allen, 1944, is a damaging parasite of *Atriplex confertifolia* (Torr. & Frem.) S. Wats. in the desert foothills of Utah. *N. batatiformis* Thorne & Schuster 1956, was first observed on sugar beet in Nebraska and can also reproduce on thirty-one plants in nine families. It has also been found on sugar beet in Colorado and Wyoming.

N. serendipiticus is distinguished from *N. dorsalis* by the absence of an elongated posterior region of the body in the mature female and, in the young female, by the shorter vulva-anus distance, which in *N. dorsalis* is three times the tail length. In *N. dorsalis* the male tail is longer and more pointed and the bursa smaller than in the new species. The larva of *N. dorsalis* is 0.5 mm long as compared with 0.3 mm in *N. serendipiticus*.

In *N. aberrans* the pre-bulbar region of the oesophagus in the female is much less developed than in the new species and the phasmids in the young female are near the tip of the tail. In the male the bursa starts just in front of the anus, whereas it starts about the level of the spicule heads in *N. serendipiticus*. The larva also differs from that of the new species in being larger (0.4-0.48 mm long and 20-24 μ wide) and having a relatively shorter tail (only twice the anal body width compared with 2.6 times in *N. serendipiticus*).

The new species is closest to *N. batatiformis*. The female differs in having a longer stylet (15-18 μ in *N. batatiformis*) and smaller eggs (49 μ × 83 μ in *N. batatiformis*). In the young female of *N. serendipiticus* the vulva-anus distance is a little greater than the tail length but is equal to it in *N. batatiformis*, and the phasmids are close behind the anus but midway along the tail in the latter species. The males of *N. batatiformis* are a little longer and more slender than in the new species (0.8-1.2 mm long and a = 32 in *N. batatiformis*) but are similar in other respects. The larvae differ in being more slender and having longer tails in the new species (a = 18, c = 15 in *N. batatiformis*) : the post-bulbar region of the oesophagus is proportionately longer, the intestine starting in front of the excretory pore in *batatiformis*. The lateral field is marked by two bright lines in *N. batatiformis* but has four clearly distinguishable incisures in *N. serendipiticus*.

Life History

The life history of *N. serendipiticus* has not yet been determined but

may resemble that of *N. batatiformis* in having two periods of parasitism. The only forms found in roots were females ranging from young cylindrical forms about 20-25 times as long as broad and without fully developed eggs, to fully mature, irregularly spindle-shaped, ovipositing forms. Numerous eggs were found in the gelatinous egg sacs but neither young nor developing larvae were found in the roots. The absence of young males from the galls suggests that, as with *N. batatiformis*, the pre-adult males may leave the roots and complete their development in the soil.

Bionomics

N. serendipiticus is known only from glasshouse tomatoes at one locality in England. It is thought to have been present there for several years but has not been a serious pest, probably because control measures against root-knot disease have been taken from time to time. In pot tests in a cool greenhouse, mangold, lettuce and *Solanum nigrum* were found with small galls containing young females in May, when the plants had been growing in the infested soil for eight months.

Diagnosis of N. serendipiticus

Nacobbus with mature females irregularly spindle-shaped without elongated posterior region. Vulva-anus distance in young females about 1½ times tail length and phasmids ad-anal or immediately post-anal. Oesophagus well-developed with corpus about half the width of muscular bulb. Stylet 19-22 μ with conspicuous knobs. Males with tylenchoid spicules and small bursa surrounding tail, the latter about as long as the anal body diameter. Phasmids appearing as ribs in bursa midway along tail. Newly hatched larvae with blunt tails and four incisures on lateral field; phasmids midway along tail. Eggs 80.4 × 37.7 μ.

Type host: *Lycopersicon esculentum* Mill.
Type locality: nursery at Wokingham, Berkshire.
Type collection: 59/4/TC. Collected by C. W. Graham, National Agricultural Advisory Service, Reading.

Key to species of Nacobbus, *adapted from* Thorne & Schuster, 1956.

1. Adult female with short neck, swollen spheroid or ovate body followed by a slender elongated posterior region; infective female with vulva-anus distance equal to 3 times tail length; male tail about 1½ times anal body diameter . . . . . *dorsalis* Thorne & Allen, 1944

Adult female with short neck and swollen spindle-shaped or irregular body but without elongated posterior region; infective female with vulva-anus distance one to two times the tail length; male tail about equal to anal body diameter 2

2. Corpus not strongly-developed in adult female; in infective female vulva-anus distance twice tail length and phasmids near tail tip . . . *aberrans* Thorne, 1935
Strongly-developed oesophageal corpus and median bulb in adult female . 3

3. In young female phasmids near middle of tail; spermatheca in mature female; female stylet 15-18 μ long; in larva lateral field marked by two bright lines, a = 18, c = 15; eggs 83 × 49 μ . *batatiformis* Thorne & Schuster, 1956

In young female phasmids ad-anal or closely post-anal; no spermatheca in mature female; female stylet 20 μ; in larva lateral field marked by four incisures, a = 23, c = 13; eggs 80 × 38 μ . . . . . *serendipiticus* n.sp.

ZUSAMMENFASSUNG

Nacobbus serendipiticus *n.sp. ein Wurzelgallen-Nematode an Tomaten in England.*

Nacobbus serendipiticus n.sp. verursacht Gallen an Tomatenwurzeln. Er unterscheidet sich von *N. dorsalis* durch die Form des reifen Weibchens, den Schwanz des Männchens und die Lage der Vulva beim jungen Weibchen. Von *N. aberrans* weicht er in der Grösse des Oesophagus beim reifen Weibchen und der Lage der Phasmiden beim jungen Weibchen ab. Von *N. batatiformis* unterscheidet er sich durch das Fehlen einer Spermatheca beim reifen Weibchen, die Lage der Phasmiden beim jungen Weibchen, die Grösse der Eier, die Länge des Oesophagus und die Form des Seitenfeldes bei den Larven.

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MISCELLANEOUS NOTES ON THE GENUS TYLENCHORHYNCHUS (TYLENCHINAE: NEMATODA)

BY

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The nematode genus *Tylenchorhynchus* Cobb, 1913 has recently been revised by ALLEN (1955). Additional new species have been described by FIELDING (1956), BROWN (1956), PAETZOLD (1958) and HOPPER (1959). In the course of the last years further data were collected, which are presented below.

DESCRIPTION OF TWO NEW SPECIES

Tylenchorhynchus bursifer n. sp. (Fig. 1A-D).

Measurements (specimens fixed in F.A.A. and mounted in glycerine) :

Females (n = 13) : L = 557-655 μ; a = 24,8-29,9; b = 4,3-5,1; c = 13,0-16,9; V = 53,5-56,3%; G_1 = 19,9-31,7%; G_2 = 23,9-29,1%; stylet = 22-24 μ.

Males (n = 7) : L = 484-618 μ; a = 24,6-28,4; b = 4,4-5,0; c = 11,4-14,2; T = 54,9-64,2%; stylet = 22-23 μ.

Female (holotype) : L = 631 μ; a = 24,8; b = 5,0; c = 13,0; V = 53,7%; G_1 = 31,7%; G_2 = 28,8%; stylet = 22 μ.

Male (allotype) : L = 589 μ; a = 27,0; b = 4,6; c = 13,5; T = 57,5%; stylet = 23 μ.

Body assuming an open C-shape when killed by gentle heat; generally slightly twisted to submedian position anteriorly; tapering distinctly towards anterior, but only little towards posterior end. Cuticular annulation distinct, moderately coarse (striae 1,4-1,5 μ apart), growing coarser towards head end (up to 2 μ). No longitudinal striae. Lateral field with four incisures, the inner ones coalescing at level of median bulb. Lip region continuous with body contour, anterior margin convex, bearing about five rather indistinct annules. Amphid apertures rather easily visible from median view, being situated in two slight depressions in anterior margin of lip region. Labial sclerotization weakly

developed, except for spear guiding apparatus. Stylet slender, with distinct, slightly obliquely flattened basal knobs. Orifice of dorsal oesophageal gland 3 μ behind spear base. Median bulb somewhat elongate; isthmus long and narrow; basal bulb pyriform, not overlapping intestine. Excretory pore opens in region of posterior half of isthmus. Hemizonid about 3 μ long, situated 1-4 annules in front of excretory pore. Intestine without postanal extension.

Female: Ovaries outstretched, oocytes in single or partly multiple row; spermathecae broadly oval. Tail suddenly constricted ventrally before middle, distal part hook-shaped, with "bursa" formed by lateral field. From this feature the specific name is derived. Phasmids conspicuous, opening in anterior third of tail.

Male: Tail of normal shape. Bursa broad. Phasmids distinct, extend into bursa, situated in anterior third of tail. Spicules 23 μ long; gubernaculum 10 μ long, hooked proximally.

Larvae: Tail shape as in adult female.

Diagnosis: *Tylenchorhynchus* differring in female from all known species by peculiar tail shape. Males resembling those of *T. parvus* Allen, 1955 and *T. striatus* Allen, 1955, but having a longer stylet and a relatively longer tail. Because the males show no deviation from the ordinary *Tylenchorhynchus* type, and the females only in one respect, this species is included in the genus *Tylenchorhynchus*.

Holotype: female in slide q 7; *allotype*: male in slide q 4; *paratypes*: 12 females and 6 males in slides q 1, q 2, q 4, q 6, q 7 and q 8. All types in the nematode collection of the Plantenziektenkundige Dienst, Wageningen.

Type locality: Boskoop, The Netherlands.

Type habitat: Soil from roots of *Rosa* sp.

Tylenchorhynchus bursifer is known only from the type locality.

Tylenchorhynchus microphasmis n.sp. (Fig. 1 E-G).

Measurements (specimens fixed in F.A.A. and mounted in glycerine):

Females (n = 11): L = 770-935 μ; a = 30,6-38,6; b = 5,2-6,1; c = 14,5-17,5; V = 48,9-55,0%; G_1 = 14,6-25,3%; G_2 = 14,6-25,6%; stylet = 24-27 μ.

Males (n = 3): L = 687-891 μ; a = 31,5-36,8; b = 4,9-5,7; c = 11,8-14,7; T = 52,8-58,2%; stylet = 24-26 μ.

Female (holotype): L = 918 μ; a = 34,5; b = 6,1; c = 15,2; V = 51,2%; G_1 = 14,6%; G_2 = 14,6%; stylet = 25 μ.
Male (allotype): L = 891 μ; a = 36,8; b = 5,6; c = 13,4; T = 54,3%; stylet = 26 μ.

Body assuming an open C-shape when killed by gentle heat; tapering distinctly towards both extremities, especially towards tail end. Cuticular

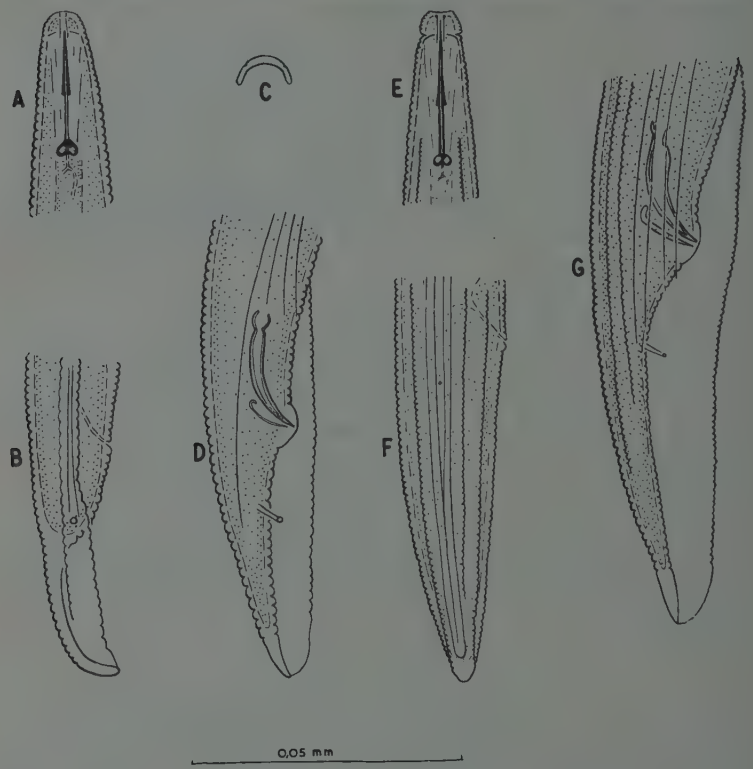

Fig. 1. *Tylenchorhynchus bursifer* n.sp. A: Head end of female, lateral view; B: Female tail, lateral view; C: Cross-section through distal part of female tail; D: Male tail, lateral view.
Tylenchorhynchus microphasmis n.sp. E: Head end of female, lateral view; F: Female tail, lateral view; G: Male tail, lateral view.

annulation fine, rather indistinct (striae 0,8-0,9 μ apart). Longitudinal striae present, very much like those of *T. lamelliferus* (De Man, 1880), numbering 16-20. Lateral field with four incisures; anteriorly the inner incisures coalesce just behind the posterior edge of the median bulb;

the resulting single incisure vanishes about halfway between median bulb and spear base. Posteriorly the inner incisures coalesce behind the phasmid (Fig. 1 F).

Lip region set off by constriction, bearing 6-7 annules; labial sclerotization inconspicuous. Stylet slender, with small but distinct basal knobs. Orifice of dorsal oesophageal gland about 3 μ behind spear base. Median oesophageal bulb large, spherical; isthmus slender; basal bulb slightly elongate. Excretory pore opens in region of anterior end of basal bulb. Hemizonid rather inconspicuous, situated 2-5 annules in front of excretory pore. Intestinal granules large.

Female: Ovaries outstretched, oocytes in single row. Spermathecae almost round. Tail conical, tapering to blunt smooth tip. Phasmids small and inconspicuous; only in one female were both visible, situated in the anterior quarter of the tail, the right one 4 μ, the left one 12 μ posterior to the anus.

Male: Similar to female. Tail very slender, pointed. Bursal edge straight; bursa envelops tail. Spicula curved, 29 μ long. Gubernaculum 14 μ long, curved proximally. Phasmids inconspicuous, only one seen; situated in anterior third of tail and extending only slightly into bursa.

Diagnosis: *Tylenchorhynchus* keying out (using ALLEN's key) with *T. lamelliferus* (De Man, 1880), but differing from the latter by its off-set lip region, slenderer body, long tapering tail and straight bursal edge. The outstanding feature of *T. microphasmis* is the smallness of the phasmids, from which the specific name was derived (coined analogous to *macramphis*).

Holotype: female in slide v 1; *allotype*: male in slide v 2; *paratypes*: 9 females and 2 males in slides v 3, v 4 and v 5. All types in the nematode collection of the Plantenziektenkundige Dienst, Wageningen.

Type locality: Westerbork, The Netherlands.

Type habitat: sandy soil.

The species was also found in soil around the roots of nettle at Hulst, in soil around roots of radish and spinach at Bennekom and in a tree nursery at Tegelen. A larva which might belong to this species was found in a soil sample from *Cocos nucifera* from the Belgian Congo.

ON THE GEOGRAPHICAL DISTRIBUTION OF TYLENCHORHYNCHUS SPECIES

According to ALLEN only two species (*T. maximus* Allen, 1955 and *T. brevidens* Allen, 1955) had up to 1955 been found on both sides

of the Atlantic, while 18 species were known from North America only, 10 from Europe only and 4 from Central Asia only. Since 1955 some new data have come to light, which show that, while there is undoubtedly a large difference between the *Tylenchorhynchus* fauna of the Old and the New World, the number of species occurring in both is greater than ALLEN supposed.

Five species known only from North America have been discovered in the Old World:

1. *T. claytoni* Steiner, 1937. This species was recorded by VOGEL and BERNET (1958) as a pest of Azaleas in Switzerland. It was also collected by Miss J. BOONSTRA in grass at the Municipal Park at Basel and around roots of *Sedum sempervivum* at Pontresina (altitude 2.100 m) and is thus probably indigenous to Switzerland. Its occurrence in the Netherlands was reported by OOSTENBRINK (1959). The present author found it in several localities in this country, viz. soil around roots of *Rhododendron* sp., Oegstgeest; soil around roots of holly, Ede [1]); waste land being brought into culture, Vessem (numerous); nurseries at Aalsmeer, Boskoop and Tegelen. Thus *T. claytoni* is probably a native of this country as well. The record from Hungary (ANDRÁSSY, 1952) is dubious.

2. *T. lenorus* Brown, 1956. A large population of this species was found around roots of strawberries at Huissen, near Arnhem, The Netherlands. In addition, one female was collected from roots of oats at Brummen, The Netherlands.

3. *T. clarus* Allen, 1955. Two females were found in clay soil at St. Annaland, Island of Tholen, in the Zeeland estuarine district in the southwestern part of the Netherlands.

4. *T. acti* Hopper, 1959. Collected by Miss BOONSTRA around roots of *Phoenix dactylifera*, Chiavari, Italy.

5. *T. nudus* Allen, 1955. Found in soil around roots of cocoa and coconut, Ceylon.

Two species known previously only from Europe have since been reported from the U.S.A.:

1. *T. dubius* (Bütschli, 1873). There are two recent records of this species from America. REYNOLDS and EVANS (1953) mention it as very common in the southwestern U.S.A. However, ALLEN (1955) stated

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1) Recently the species was also recorded from Ede (soil around roots of *Kalmia*) by SEINHORST (1958).

that *T. dubius* had not yet been found in the U.S.A., so this record most probably relates to some other species.

The second record is of 1957, i.e. after the publication of ALLEN's revision. JENKINS, TAYLOR, ROHDE and COURSEN report *T. dubius* as occurring frequently in the Northern and Western counties of Maryland. It is remarkable that the species occurs there on clay-loam type soils, whereas in the Netherlands it is found almost exclusively in light sandy soils. This might perhaps be a temperature influence.

2. *T. ornatus* Allen, 1955. Reported by JENKINS, TAYLOR, ROHDE and COURSEN (1957) from Maryland, but found in one county only.

Thus of the total number of 46 known *Tylenchorhynchus* species nine occur in both the Old and the New World. It might be noted that all species possessing longitudinal cuticular striation are known from Europe. There are, however, apparently no reasons to regard this group of species more closely related to each other than to the species without longitudinal striae.

A NOTE ON THE NOMENCLATURE OF TYLENCHORHYNCHUS MACRURUS

According to ALLEN (1955), *T. macrurus* (Goodey, 1932) occurs in a smaller and a larger form. The status of these forms has not yet been clarified, but we may note that the small form has been described by STEINER (1914) as *Aphelenchus dubius*; perhaps *Tylenchorhynchus graminicola* Kirjanova, 1951 is also identical with this form. Since a lectotype has not yet been selected, the name *macrurus* is not yet attached to either the small or the large form.

MORPHOLOGICAL NOTES ON SOME SPECIES

Tylenchorhynchus acti Hopper, 1959.

The material obtained from Italy consisted of fourteen females. Measurements (specimens fixed in F.A.A. and mounted in glycerine): L = 631-830 μ; a = 31,0-37,6; b = 4,7-5.5; c = 14,3-17,4; V = 53,5-58,0%; G_1 = 20,9-25,7%; G_2 = 21,5-25,2%; stylet = 16-18 μ.

To HOPPER's description the following points are added:

Body assuming a loose spiral shape when killed by gentle heat, as in *T. maximus* Allen, 1955 and *T. macrurus* (Goodey, 1932). Hemizonid weakly developed, invisible in most specimens, in others appearing as a small clear streak. Tail end variable: rounded in some specimens, angular in others, but always with the characteristic enlarged tip. The

mean number of tail annules was 41 (range 32-35). Phasmids situated in the anterior two-fifths of the tail, usually opposite each other; in a few specimens, however, the left one was situated slightly more posteriorly than the right one. Spermathecae apparently absent.

Tylenchorhynchus nudus Allen, 1955.

The material obtained from Ceylon consisted of three males, seven females and a few larvae. Measurements (specimens fixed in F.A.A. and mounted in glycerine):

Females: L = 563-666 μ; a = 28,1-30,5; b = 4,7-5,3; c = 12,2-15,3; V = 53,4-56,2%; G_1 = 22,7-27,4%; G_2 = 19,7-25,7%; stylet = 17-21 μ.

Males : L = 518-572 μ; a = 24,8-32,9; b = 4,3-4,9; c = 14,3-15,7; T = 45,8-62,4%; stylet = 18-20 μ.

Body curved only slightly when killed by gentle heat. Annulation of cuticle very coarse (distance between striae about 1,9 μ), especially on the distal half of the tail. Hemizonid immediately anterior to excretory pore, linear, 3-3,5 μ long.

Female: Ovaries with well developed, round or broadly oval spermathecae, containing spermatozoa. Tail generally more slender than illustrated by Allen's fig. 3 J, and more narrowly rounded (Fig. 2). The two inner lateral incisures coalesce behind the phasmid, whereas Allen draws them as separate over the whole length of the tail. The number of tail annules varied in the Ceylonese specimens from 15 to 22. Phasmids on anterior third of tail; in three specimens they were located opposite each other, in four the left was situated more posteriorly than the right.

Male: agreed with Allen's description.

From the foregoing it is clear that the female specimens differ in some minor respects from the American type material. The Ceylonese specimens are also notably smaller in size; this may, however, be partly the effect of fixation and mounting. In the writer's opinion the differences are not significant enough to warrant the view that the Ceylonese specimens are specifically different from the American ones. Allen found males, thus his females may have possessed spermathecae, although he was not able to see them distinctly.

According to Hopper (1959), *T. ewingi* Hopper, 1959 differs from *T. nudus* by shorter male tail (36-46 μ against 50 μ) and by the presence of spermathecae. These differences do not hold; the tails of the

Ceylonese males were 34-40 μ in length. The number of lip annules and the presence in *T. ewingi* of a postanal intestinal blind sac seem good differentiating characters.

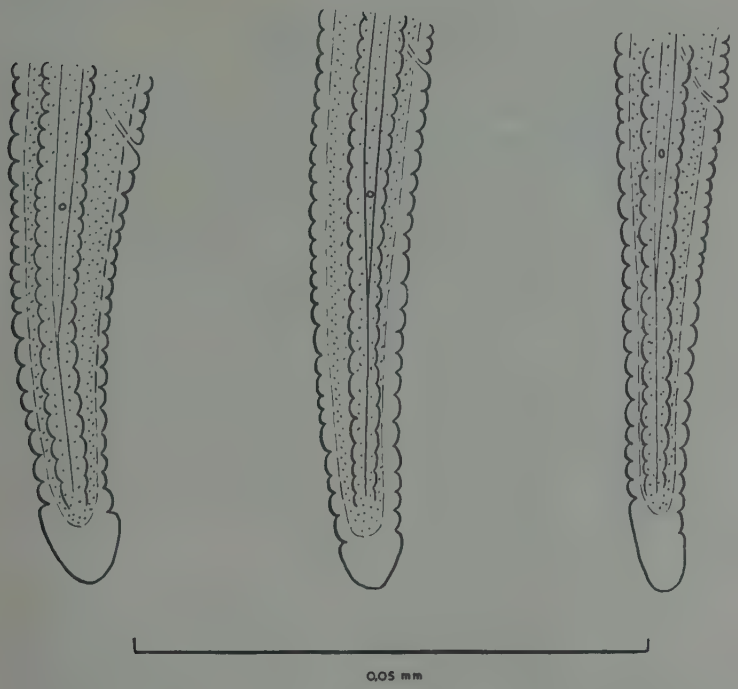

Fig. 2. *Tylenchorhynchus nudus* Allen. Variation in female tail shape.

KEY TO TYLENCHORHYNCHUS

The following key is essentially an emendation of that given by ALLEN (1955). The new species described since 1955 have been inserted, together with some Russian species not considered by ALLEN. The descriptions of two Russian species were not accessible, viz. *T. bucharius* (Tulaganov, 1949) Tulaganov, 1954 and *T. caromatus* (Tulaganov, 1949) Tulagonov 1954.

In the writer's opinion *Tetylenchus dimidius* Kirjanova, 1951 belongs in the genus *Tylenchorhynchus*, since the illustration shows the female tail to be broadly rounded, but the description does not contain sufficient information to permit insertion of this species into the key.

1.	Stylet very short (10 μ); female tail mucronate	*paucus* Kirjanova, 1951.
	Stylet usually longer than 10 μ; female tail not mucronate . . .	2.
2.	Cuticula with longitudinal striation	3.
	Cuticula without longitudinal striation	9.
3.	Lateral field with 4 incisures . .	4.
	Lateral field with 6 incisures . .	6.
4.	Annulation coarse (about 2 μ); lip region with 3-4 annules . . .	*claytoni* Steiner, 1937.
	Annulation fine (1 μ or less); lip region with 6-7 annules	5.
5.	Lip region continuous; tail short .	*lamelliferus* (De Man, 1880).
	Lip region set off by constriction; tail long	*microphasmis* n.sp.
6.	Tail tip annulated	*tessellatus* Goodey, 1952.
	Tail tip smooth	7.
7.	About 60 longitudinal striae on middle of body	*quadrifer* Andrássy, 1954.
	No more than about 32 longitudinal striae on middle of body . . .	8.
8.	Lip region set off; 24 longitudinal striae	*lenorus* Brown, 1956.
	Lip region continuous; 32 longitudinal striae	*ornatus* Allen, 1955.
9.	Lateral field with 4 incisures . .	10.
	Lateral field with 5 incisures . .	27.
	Lateral field with 6 incisures . .	29.
10.	Female tail hook-shaped; with "bursa"	*bursifer* n.sp.
	Female tail of normal shape . . .	11.
11.	Tail tip annulated	12.
	Tail tip smooth	18.
12.	Lip region set off by construction	*dubius* (Bütschli, 1873).
	Lip region continuous	13.
13.	Lip region with 4-5 annules . . .	14.
	Lip region with 6-8 annules; female tail broadly rounded . .	15.
14.	Tail conoid, rather narrowly	

	rounded	*eremicolus* Allen, 1955.
	Tail subcylindrical, broadly rounded	*hüsingi* Paetzold, 1958.
15.	Lip sclerotization heavy, conspicuous	16.
	Lip sclerotization faint, inconspicuous	17.
16.	Tail very short (c over 30)	*brevicaudatus* Hopper, 1959.
	Tail of normal length (c under 20)	*magnicauda* (Thorne, 1935).
17.	Tail length less than 3 times anal body diameter	*parvus* Allen, 1955.
	Tail length more than 3 times anal body diameter	*maximus* Allen, 1955.
18.	Lip region continuous with body contour	19.
	Lip region set off by constriction or depression	25.
19.	Lip region with 2 annules	*nudus* Allen, 1955.
	Lip region with more than 2 annules	20.
20.	Lip sclerotization faint, inconspicuous	21.
	Lip sclerotization heavy, conspicuous	23.
21.	Tail with more than 20 annules	*striatus* Allen, 1955.
	Tail with less than 15 annules	22.
22.	Lip region with 5 annules; intestine without postanal extension	*clarus* Allen, 1955.
	Lip region with 3-4 annules; intestine with postanal extension	*ewingi* Hopper, 1959.
23.	Stylet length less than 20 μ	*manubriatus* Litvinova, 1946.
	Stylet length more than 20 μ	24.
24.	Stylet length 28-31 μ; tail with about 50 annules	*kegenicus* Litvinova, 1946.
	Stylet length 31-39 μ; tail with about 45 annules	*galeatus* Litvinova, 1946.
25.	Stylet length more than 20 μ; lip sclerotization heavy	*cylindricus* Cobb, 1913.
	Stylet length less than 20 μ; lip sclerotization faint	26.

26.	Lip region with 3 annules; tail with more than 18 annules	*martini* Fielding, 1956.
	Lip region with 6 annules; tail with less than 18 annules	*latus* Allen, 1955.
27.	Tail length 2 x anal body diameter; tail with less than 25 annules . .	*acutus* Allen, 1955.
	Tail length 3 x anal body diameter; tail with more than 25 annules .	28.
28.	Lip region set off distinctly; tail tip not enlarged	*capitatus* Allen, 1955.
	Lip region nearly continuous; tail tip enlarged	*acti* Hopper, 1959.
29.	Tail tip annulated	30.
	Tail tip smooth	35.
30.	Lip region set off by constriction or depression	31.
	Lip region continuous	32.
31.	Lip region set off by constriction; stylet length less than 23 μ . .	*bogdanovi-katjkovi* (Kirjanova, 1941).
	Lip region set off by depression; stylet longer than 23 μ	*leptus* Allen, 1955.
32.	Lip sclerotization heavy, conspicuous	*macrurus*, (Goodey, 1932).
	Lip sclerotization faint, inconspicuous	33.
33.	Stylet length more than 23 μ . .	*obscurus* Allen, 1955.
	Stylet length 22 μ or less	34.
34.	Stylet length 16-18 μ; tail length less than 3 x anal body diameter	*nothus* Allen, 1955.
	Stylet length 11-15 μ; tail length more than 3 x anal body diameter	*nanus* Allen, 1955.
35.	Lip region continuous with body contour	36.
	Lip region set off by constriction or depression	38.
36.	Tail short, conoid, terminus pointed	*brachycephalus* Litvinova, 1946.
	Tail subcylindrical, bluntly rounded	37.
37.	Stylet length less than 20 μ . . .	*brevidens* Allen, 1955.
	Stylet length more than 20 μ. . .	*affinis* Allen, 1955.

38. Lip sclerotization heavy, conspicuous . . . . . 39.
Lip sclerotization faint, inconspicuous . . . . . 41.
39. Tail length more than 3 x anal body diameter . . . . . *alpinus* Allen, 1955.
Tail length less than 3 x anal body diameter . . . . . 40.
40. Stylet length more than 35 μ. . . *macrodens* Allen, 1955.
Stylet length less than 35 μ. . . . *grandis* Allen, 1955.
41. Stylet length less than 35 μ. . . . *lineatus* Allen, 1955.
Stylet length more than 35 μ. . . 42.
42. Stylet length more than 55 μ. . . *superbus* Allen, 1955.
Stylet length less than 55 μ. . . . *conicus* Allen, 1955.

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ZUSAMMENFASSUNG

Bemerkungen zu der Gattung Tylenchorhynchus (*Nematoda*: *Tylenchinae*).

Aus der Gattung *Tylenchorhynchus* werden zwei neue Arten beschrieben: *T. bursifer* n.sp., hauptsächlich charakterisiert durch den eigentümlichen Bau des weiblichen Schwanzes, und *T. microphasmis* n.sp., der *T. lamelliferus* (De Man) nahesteht, sich jedoch von dieser Art unterscheidet durch die abgesetzte Lippengegend und den langen Schwanz.

Weiterhin enthält diese Arbeit neue Daten über die geographische Verbreitung einiger Arten, einige Bemerkungen zur Nomenklatur von *T. macrurus* (Goodey) und einige morphologische Notizen über *T. nudus* Allen und *T. acti* Hopper. Schliesslich wird eine Bestimmungstabelle gegeben, basiert auf jene von Allen (1955), die die seither neubeschriebenen Arten einschliesst, soweit die Beschreibungen zugänglich waren.

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THE EFFECT OF GROWING RESISTANT POTATOES ON A POTATO ROOT EELWORM (*HETERODERA ROSTOCHIENSIS* WOLL.) POPULATION

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Eelworm-resistant potatoes bred from *Solanum tuberosum* subsp. *andigena* clones C.P.C. 1673, 1685 and 1690 produce a root diffusate with a hatching power about equal to that of standard commercial varieties, are invaded by larvae in numbers comparable to those invading the standard varieties, but have either no or very few cysts on their roots when grown in many infested soils (JONES, 1954; WILLIAMS, 1958). The growing of such resistant potatoes thus produces a marked decrease in the number of viable eggs in such soils (JONES, 1954; HUIJSMAN, 1957; WILLIAMS, 1958). There are, however, soils which contain a type of potato root eelworm (*Heterodera rostochiensis* Woll.) capable of producing cysts on resistant material bred from clones C.P.C. 1673, 1685 and 1690 (DUNNETT, 1957; JONES, 1957, 1958 and 1959; HOWARD, 1959). It is therefore of importance to know whether the continued growing of resistant potatoes will lead to an increase in the frequency of "resistance-breaking" eelworms, and how rapidly any increase will occur in soils which initially are free from or have only a low frequency of "resistance-breaking" types.

THE FIELD PLOTS

The results given in this paper are for field plots on a farm in Feltwell Fens, Norfolk, near Little Ouse. The soil is a black fen soil. These plots had been used extensively by Dr. T. D. Williams in his investigations (WILLIAMS, 1958) before they were taken over by the Plant Breeding Institute in 1958. There are 42 plots in all, arranged in six blocks of seven plots, each plot being six feet (about two metres) square and each being planted every year with nine potatoes (three rows of three). There are five paths, four and a half feet (about one and a half

metres) wide, between the six blocks and these are not cropped. Previous to 1955, the cropping of the field was: — 1947, Carrots; 1948, Potatoes; 1949, Rye; 1950, Carrots; 1951, Potatoes; 1952, Carrots; 1953, Potatoes; and 1954, Rye.

RESULTS FOR 1955-57

The results of soil population counts for the period 1955-57, made by Williams are summarised in Table I. To Williams' results have been added an eighth set for fallow (i.e. no crop) for three years. The ten soil samples for these were obtained from the five paths, two positions

TABLE I

Effect of the repeated growing of resistant potatoes on the eelworm population (soil counts by T. D. Williams and F. G. W. Jones).

Cropping[1]		Eelworm populations (air dried soil)[2] Initial 1955		Final 1957		Eggs
1955	1956 & 1957	Cysts/ 100 gm.	Eggs/ gm.	Cysts/ 100 gm.	Eggs/ gm.	Final 1957/ Initial 1955
Majestic	Z3/7	43	75	161	133	175%
U. Chieftain	„	46	67	68	19	28%
Fallow	„	55	88	51	11	12%
S. nigrum	„	46	94	58	11	11%
Z 3/2	„	56	91	59	17	18%
Z 3/7	„	45	76	44	9	11%
Z 17/2	„	50	79	47	11	14%
Fallow	Fallow	51	82	42	31	37%

[1] Z 3/2, Z 3/7 and Z 17/2 — resistant potatoes bred from C.P.C. 1685.

[2] Populations — mean of 6 plots per treatment, except for fallow — Z 3/7 which is a mean of 9, Z 3/2 — Z 3/7 which is a mean of 3, and fallow — fallow which is a mean of 10.

in each path being sampled. The initial values for the eelworm populations of these ten path positions were calculated as the means between the two initial values for the plots on either side of them. The final 1957 values were determined by Mr. F. G. W. Jones of Rothamsted from samples collected by us.

The results given in Table I show that the growing of resistant potatoes for three successive years has resulted in a bigger reduction in the egg population than has three years fallow. On the other hand two years of resistant potatoes after a crop of the susceptible variety Majestic still shows a high population. The low value for Ulster Chieftain followed by two years resistant potatoes is presumably due to the Ulster Chieftain making very poor growth in 1955.

The first experiment to determine whether the continued growing of resistant potatoes had led to an increase in the frequency of "resistance-breaking" eelworms was made in the spring of 1958, i.e. with the soils in the conditions they would be in after growing the 1957 crops. Samples were taken from all the 42 plots (seven treatments, six plots for each treatment) and from the ten path positions. Two pots of 3½ inches (9 cm) diameter size were used from each plot, one planted with Arran Pilot to determine the total cyst-producing ability and one with X59/1 (a resistant clone bred from C.P.C. 1690) to determine the frequency of "resistance-breaking" types (Jones, 1957, 1958 and 1959; Howard, 1959). The number of cysts on the outside of the root ball was counted for each pot. The results are summarised in Table II.

Table II

Results of the 1958 pot experiment

Cropping[1]		Average number of cysts per pot on	
1955	1956 and 1957	Arran Pilot	X 59/1[2]
Majestic	Z 3/7	230	0.1
U. Chieftain	„	130	0.0
Fallow	„	60	0.2
S. nigrum	„	60	0.0
Z 3/2	„	50	0.3
Z 3/7	„	40	0.5
Z 17/2	„	50	0.0
Fallow	Fallow	140	0.6

[1] See Table I.
[2] X 59/1 — resistant potato bred from C.P.C. 1690.

In Table II there is no indication that the growing of resistant potatoes for two or three years leads in this particular soil to any increase in the frequency of "resistance-breaking" types of eelworm. The results can be stated in another form. For the three years fallow (ten samples), there were found 1,391 cysts on the roots of Arran Pilot and six on the roots of X59/1; for the two or three years resistant potatoes (42 samples), there were found a total of 3,611 cysts on Arran Pilot and seven on the roots of X59/1. It must, however, be pointed out that the resistant potatoes grown in the field (Z3/2, Z3/7 and Z17/2) were bred from clone C.P.C. 1685 while the resistant potato used in the 1958 pot test, X59/1, was bred from clone C.P.C. 1690, and that there is some indication (Howard, 1959), that the clones C.P.C. 1685 and 1690 do not necessarily contain the same resistance gene. The results for the 1959 pot experiment, see Tables IV-VI, on the other hand suggest that

the "resistance-breaking" eelworms in this population are equally effective in material bred from either C.P.C. 1673, 1685 or 1690.

The results given in Tables I and II are summarised and compared in Table III. In the last Table the fallow and *S. nigrum* treatments of 1955 have been grouped together as fallow because the *S. nigrum* only produced very small plants which could not have had much effect on the eelworm population. The three resistant potato treatments of 1955, have also been combined. With the exception of the value for

TABLE III

Changes in the egg populations with cropping. Comparisons of expected with observed values from both soil counts and pot experiment

Treatment	Eggs Final 1957/Initial 1955		
	Expected[1]	Soil Counts	1958 pot experiment
Majestic + 2 years resistant potatoes	150%	175%	67%
U. Chieftain + 2 years resistant potatoes	38%	28%	37%
Fallow + 2 years resistant potatoes	19%	12%	16%
3 years resistant potatoes	13%	14%	(13%)[2]
3 years fallow	42%	37%	40%

[1] Expected calculated using the following values: — Majestic, 6-fold increase; U. Chieftain, 1½-fold increase; fallow, 25% reduction each year; and resistant potatoes, 50% reduction each year.

[2] In the 1958 pot experiment a value of 13% has been assumed for three years resistant potatoes in order to calculate the other figures in this column.

Majestic plus two years resistant potatoes, there is a close agreement between the expected values and those found from both the soil counts and the 1958 pot experiment results. The low value for the Majestic plus two years of resistant potatoes in the 1958 pot experiment may be due to some extent to there not being sufficient Arran Pilot roots in the small pots for all the potential cysts to develop.

RESULTS FOR 1958

In 1958 the plots were planted with several different resistant clones. These were: — Y 12/37 (ex C.P.C. 1685), Y 56/103 (ex C.P.C. 1690), X53/17 (ex C.P.C. 1673), X10/11 (ex C.P.C. 1685), X8/5 and X8/18 (ex C.P.C. 1673), and X55/33 and X55/13 (ex C.P.C. 1685). This wider range of resistant material has somewhat complicated the plot treatments, see Table V.

A second pot experiment was carried out in the spring of 1959,

i.e. on the soils as they would be after the 1958 growing season. This was similar to the 1958 pot experiment, but resistant plants bred from all three *andigena* clones (*i.e.* C.P.C.'s 1673, 1685 and 1690) were used this time. The results are given in Tables IV, V and VI. The most important point shown by the results is the increase, particularly after four years of resistant potatoes, in the frequency of "resistance-breaking" eelworms.

In Table IV the agreement between the expected eelworm populations and the values calculated from the 1959 pot experiment is not as good as that found in the 1958 experiment (Table III), although the Majestic plus three years of resistant potatoes now gives a figure of the same order as the expected. Much of the lack of agreement for the other treatments is probably due to very large sampling errors when low populations of eelworms are involved. The rather high figure for four years resistant potatoes as compared with the expected may be a true value because of the increase in "resistance-breaking" types in this treatment.

TABLE IV

Results of the 1959 pot experiment

Treatments	Average number of cysts per pot on[1]				Eggs Final 1958/Initial 1955	
	A. Pilot	X53/17	Y12/37	Y56/103	Expected[2]	1959 pot experiment
Majestic + 3 years resistant potatoes	197	2.5	2.7	0.0	75%	54%
U. Chieftain + 3 years resistant potatoes	24	0.0	0.2	0.0	19%	6%
Fallow + 3 years resistant potatoes	29	1.6	0.3	1.5	9%	8%
4 years resistant potatoes	52	5.7	3.7	5.6	6%	14%
4 years fallow	116	1.1	0.2	0.6	32%	(32%)[3]

[1] X53/17, bred from C.P.C. 1673; Y12/37 from C.P.C. 1685; and Y56/103 from C.P.C. 1690.

[2] Expected populations — calculated as in Table III.

[3] A value of 32% has been assumed for 4 years fallow in calculating the other 1959 pot experiment values — actually found was 38 cysts with 2,000 eggs per 100 gm. air dried soil, i.e. 24% of the initial 1955 figure.

As can be seen from Table V the resistant potatoes grown in the four years, 1955 to 1958, have been mainly stocks bred from C.P.C. 1685. The "resistance-breaking" types of eelworm, which have been selected by the continued growing of resistant potatoes, appear, however, to be able to break the resistance equally of potatoes bred from

TABLE V

Results of the 1959 pot experiment, arranged so as to show types of resistant potatoes grown in the six blocks

Block	Types of resistant potatoes grown 1955-56-57	1958	Average number of cysts per pot on[2] A. Pilot	X53/17	Y12/37	Y56/103
T	ex C.P.C. 1685	ex C.P.C. 1685	78	1.3	0.4	0.0
U	„	ex C.P.C. 1685	37	7.6	3.0	5.9
V	„	ex C.P.C. 1690	33	0.7	0.0	2.7
W	„	ex C.P.C. 1673[1]	41	0.7	0.1	0.3
X	„	ex C.P.C. 1673	87	6.7	5.9	0.9
Y	„	ex C.P.C. 1685	127	1.4	3.4	6.3
Paths	Fallow	Fallow	116	1.1	0.2	0.6

[1] 5 plots ex C.P.C. 1673, and 2 plots ex C.P.C. 1685.
[2] X53/17, Y12/37 and Y56/103, see Table IV.

all three *andigena* clones (*i.e.* C.P.C.'s 1673, 1685 and 1690), see especially Table VI. There is thus no support from these results for the suggestion of HOWARD (1959) that the three *andigena* clones may contain different resistance genes.

There is strong evidence in Table V that the original population of eelworms in the field was not homogeneous for the occurrence of the rare "resistance-breaking" type of eelworm. Thus in blocks T, V and W there appears to have been little increase in the frequency of "resistance-breaking" eelworms which has been confined mainly to blocks U, X and Y.

The figures in Table IV, and see also Table III, appear to show that little or no increase in the frequency of "resistance-breaking" eelworms has taken place after three years of growing resistant potatoes and that it is only after four years of resistant potatoes that an increase takes place. However, if we consider individual plots (Table VI), and not average values as in Tables III and IV, it can be seen that some plots, e.g. U7, V2 and X5, have shown increases after three years of resistant potatoes. Plot U2 may also have shown an increase in the 1958 pot experiment, *i.e.* after three years of resistant potatoes. On the other hand, comparing the 1958 with the 1959 pot experiment figures of Table VI, there is still strong evidence that in most plots it has taken four crops of resistant potatoes to produce the marked increases in the frequencies of "resistance-breaking" types, although it must be recognised that in the 1959 experiment there was three times the chance of that in the 1958 experiment of finding "resistance-breaking" types due to three pots of resistant potatoes being used in

Table VI

Results for individual plots which in the 1959 pot experiment appear to show an increase in the frequency of "resistance-breaking" eelworms

Plot No.	Cropping[1] 1955	1956 & 1957	1958	1958 pot expt.[2] No. of cysts on A. Pilot	X59/1	1959 pot experiment[3] Number of cysts on A. Pilot	X53/17	Y12/37	Y56/103
T 2	*S. nigrum*	Z 3/7	X55/33	26	0	6	4	0	0
T 5	Z 17/2	„	X55/33	45	0	38	5	2	0
U 2	Z 3/7	„	X8/5	10	3	86	32	11	27
U 4	*S. nigrum*	„	X8/5	102	0	24	2	0	7
U 5	Z 3/2	„	X8/18	38	0	7	0	7	0
U 6	Z 17/2	„	X8/18	57	0	42	4	0	7
U 7	Fallow	„	X8/18	74	0	22	12	3	0
V 2	Fallow	„	X53/17	52	1	5	5	0	0
V 7	Z 3/7	„	X10/11	132	0	42	0	0	19
W 1	Z 17/2	„	Y56/103	20	0	4	4	1	1
X 2	Z 17/2	„	Y12/37	32	0	98	30	3	0
X 5	Majestic	„	Y12/37	139	0	124	15	15	0
X 6	Z 3/7	„	Y12/37	9	0	37	0	8	0
X 7	Z 3/2	„	Y12/37	72	1	291	2	14	6
Y 3	Z 17/2	„	Y12/37	71	0	36	0	2	5
Y 5	Z 3/7	„	Y12/37	31	0	49	8	7	18
Y 7	*S. nigrum*	„	Y12/37	69	0	25	0	0	14
			Total	979	5	936	123	73	104

[1] Bred from C.P.C. 1673, X8/5, X8/18 and X53/17; bred from C.P.C. 1685, Z 3/2, Z 3/7, Z 17/2, X55/33, X10/11 and Y12/37; and bred from C.P.C. 1690, Y56/103.

[2] X59/1, bred from C.P.C. 1690.

[3] X53/17, Y12/37 and Y56/103 bred from C.P.C.'s 1673, 1685 and 1690 respectively.

1959 and only one pot in 1958. The apparent increase after four crops and not after three crops of resistant potatoes is presumably related to the low frequency of "resistance-breaking" types of eelworm in the original population. It may also be explained to some extent by the hypothesis (JONES, 1959; HOWARD, 1959) that the two types of eelworm, "non-resistance-breaking" and "resistance-breaking", interbreed and that "resistance-breaking" is recessive to "non-resistance-breaking". The latter would lead to the larvae in the cysts formed by "resistance-breaking" females on the roots of resistant plants in 1955, 1956 and 1957 being mainly heterozygotes which could not form cysts on resistant plants. With the decrease in the frequency of "non-resistance-breaking" types due to growing resistant potatoes, the cysts formed in 1958 on the resistant potatoes would contain a much higher frequency of larvae homozygous for recessive "resistance-breaking". If this is so, still higher frequencies of "resistance-breaking" types of eelworm should be found after the 1959 crop of resistant potatoes. The three plots (T2, U4 and Y7), which grew *S. nigrum* in 1955, present a further problem because this species may show a similar reaction to some eelworm biotypes as do the resistant *andigena* clones (SCHICK & STELTER, 1959).

DISCUSSION

The results given in the previous sections show that the growing of resistant potatoes produces a greater decrease in the eelworm population than does fallowing (no crop). On the other hand they show that it would take many crops, even if there was no increase in "resistance-breaking" types, on this fen soil to reduce the population to a level where two crops of a susceptible variety would not raise it again to a dangerous level. It must, however, be pointed out that the figures refer to one soil type only, to cysts of one set of ages, and to one set of climatic conditions. In other soils there may be somewhat bigger reductions both from the growing of resistant potatoes and from fallow or non-host crops. This appears to be so for a sandy soil in a microplot experiment (COLE & HOWARD, unpublished), and to some extent for the sandy soil used by HUIJSMAN (1957), whose results are summarised in Table VII. Thus in Huijsman's experiment two years of resistant potatoes, two years of non-host crops, and one year of a susceptible potato plus one year of resistants gave populations similar respectively to three years of resistant potatoes, three years fallow and one year of a susceptible potato plus two years of resistants in the fen soil (Table

Table VII

Changes in egg population with cropping — from Table 6.2 of Huijsman *(1957)*

Plot No.'s	Cropping[1] 1954	Cropping[1] 1955	Eggs per gm. soil spring 1954	Eggs per gm. soil autumn 1954	Eggs per gm. soil autumn 1955	Eggs autumn 1955/ spring 1954
A1, B2	Libertas	res AT's	49	231	79	163%
C2, D3, E1	Libertas	beans	56	206	135	241%
A3, B3, C1, D2, E2	res AT's	res AT's	59	16	9	16%
A2, B1, C3, D1, E3	beans	beans	56	26	23	40%

[1] Libertas, susceptible variety; res AT's, E1 hybrids from C.P.C. 1673 crossed by commercial varieties.

III). It should, however, also be noted that the results of Huijsman (1957) — see Table VII — suggest that the decrease in eelworm populations both from growing resistant potatoes and from non-host crops is greater in the first year after susceptible potatoes than in the second.

The results for the increase in the frequency of "resistance-breaking" types of eelworm also obviously refer only to the particular soil and the cropping sequence used. With a lower initial frequency of "resistance-breaking" types their build-up would presumably be much slower. Also in actual farming practice it is to be hoped that resistant potatoes would not be grown more frequently than one year in four. There would thus be time between potato crops for the normal decrease in populations to occur, and it should therefore, even with a soil similar to the one investigated, take more than four crops of resistant potatoes to increase the frequency of "resistance-breaking" types of eelworm to a significant level. An alternation of standard susceptible varieties with resistant varieties might also delay the build-up of "resistance-breaking" types of eelworm, if "resistance-breaking" is recessive to "non-resistance-breaking" in the eelworm.

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ZUSAMMENFASSUNG

Der Einfluss resistenter Kartoffelsorten auf den Kartoffelnematoden, Heterodera rostochiensis *Woll.*

Ein Feldversuch wurde durchgeführt, um festzustellen, wie schnell die „resistenzbrechenden" Biotypen des Kartoffelnematoden (*Heterodera rostochiensis*) sich vermehren, wenn resistente Kartoffeln wiederholt auf einem Boden angebaut werden. Die Ergebnisse zeigen, dass nach dreimaligem Anbau resistenter Kartoffeln eine geringe oder keine Zunahme in der Verseuchung des „resistenzbrechenden" Biotypen eintrat, aber nach viermaligem Anbau kam es zu einer deutlichen Zunahme der „resistenzbrechenden" Biotypen. Die angebauten resistenten Kartoffeln wurden hauptsächlich aus *Solanum tuberosum* ssp. *andigena* C.P.C. 1685 gezüchtet. Die „resistenzbrechenden" Biotypen entwickelten gleich grosse Zystenmengen an Material, das auf C.P.C. 1673, C.P.C. 1685 oder C.P.C. 1690 aufgebaut ist.

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HOPLOTYLUS FEMINA N.G., N.SP. (PRATYLENCHINAE: TYLENCHIDA) ASSOCIATED WITH ORNAMENTAL TREES

BY

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During routine examination of soil and root samples from old ornamental gardens near Wageningen nematodes were observed, which represent a new tylenchid genus described below. Specimens were found around and in the roots of *Quercus robur* L. and *Chamaecyparis lawsoniana* Parl. All samples were taken from sandy soils.

Hoplotylus n.g.

Diagnosis: Pratylenchinae. Body cylindrical, tapering to tail end. Head slightly offset with heavily sclerotized framework. Lip region high, conoid, striated. Body cuticle finely annulated, except on tail tip, where striation is coarser. Stylet well developed with three oval knobs, close together; the anterior margin of each knob forms teeth. Behind median bulb the esophagus narrows to isthmus and then widens into a lobe-like glandular region, the posterior part of which overlaps intestine dorsally. Nerve ring present. Ovary single, outstretched; vulva situated ventrally in posterior part of body. Postvulval sac present. Phasmid apertures pore-like. Tail distinctly longer than anal body width.

The generic name is derived from the spear (ὅπλον = arming, τυλος = knob). Typus generis: *Hoplotylus femina* n.sp.

Hoplotylus femina n.sp.

Fig. 1.

Dimensions. 30 ♀♀: L = 664 μ (574-789); a = 23.1 (18.7-29.2); b = 6.2 (5.2-9.7); c = 18.4 (14.9-23.2); V = 87.3% (85.7-90.0); stylet = 27 μ (25-29). (freshly relaxed nematodes).

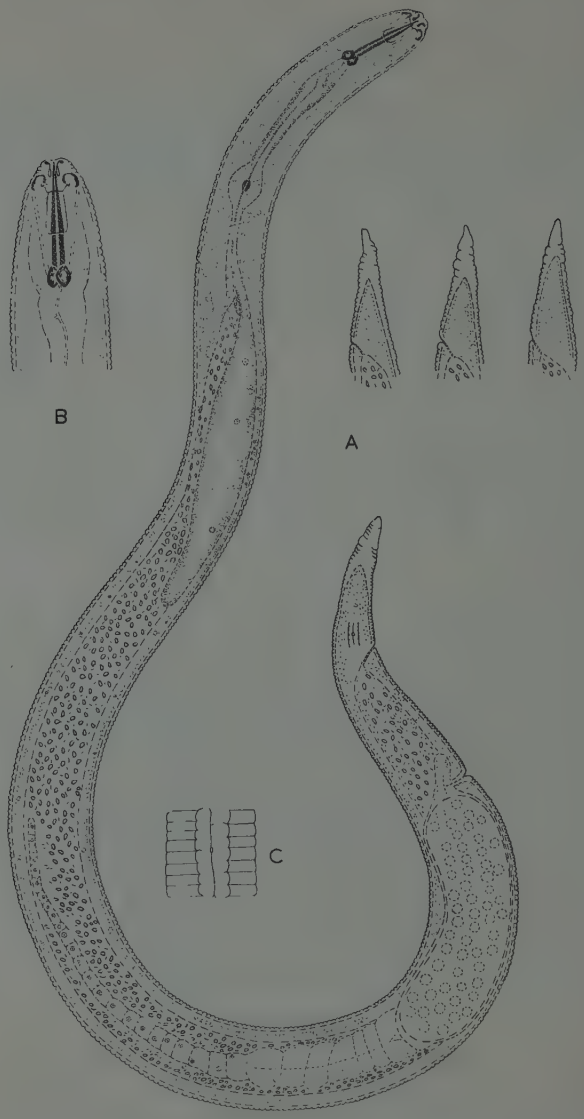

Fig. 1. *Hoplotylus femina.* A. female nematode; variations in female terminus, 500 x; B. female head, 770 x; C. lateral view of cuticle near phasmid region, 1000 x.

29 ♀♀: L = 536 μ (450-656); a = 26.2 (23.1-30.6); b = 5.9 (5.1-7.5); c = 16.1 (14.2-19.2); V = 85.9% (84.0-87.3); stylet = 25 μ (21-26). (fixed in F.A.A. and mounted in glycerine).

Female (holotype): L = 609 μ; a = 25.6; b = 5.7; c = 17.4; V = 86.0%; stylet = 23.8 μ (fixed in F.A.A. and mounted in glycerine).

Body rather stout. Lateral field with four incisures, the inner ones coalescing at anterior and posterior parts of the body. Deirids not observed. Cuticle thin, finely striated; striae about 1.2 μ apart; further apart on the tail. Lip region slightly offset from body, bearing four annules. Labial framework heavily sclerotized, yellowish; with in the centre a spear guide, forming posteriorly a blunt eggshaped extension, in which a faint ring is observed, surrounding the spear. The outer margin of the framework extends conspicuously into body about three body annules and into lip region about one annule. Spear moderately long and stout with three oval basal knobs, each of which tapers anteriorly to a dentated tip. Orifice of dorsal esophageal gland about 7 μ behind spear base. Lumen of esophagus conspicuous; beginning of intestine marked by dark cells, probably indicating the esophago-intestinal valve. Excretory pore opens near anterior end of the glandular lobe. Hemizonid present, one annule anteriorly to excretory pore. Gonad consisting of a single row of cells, in some specimens anteriorly partly double. Spermatheca or sperms not seen. Postvulval sac about half as long as vulval-body-width. Size of egg measured inside body: 75 × 18 μ (n = 1). Tail tapering, with irregular coarse striations, which do not extend around terminus. The phasmids are usually slightly posteriorly to anus and come to the surface midway the lateral field. Male unknown.

Holotype: female in slide a1 of the Plantenziektenkundige Dienst Collection, Wageningen, Netherlands.

Paratypes: 28 females in slides a2-a7 of the same collection.

Type habitat: Roots of *Quercus robur* L. and surrounding soil.

Type locality: Belmonte, Wageningen, Netherlands.

Other records: soil around roots of *Chamaecyparis lawsoniana* Parl., Wageningen and *Picea abies* L., Bussum, Netherlands. Also in soil collected at 's Graveland by Mr. J. D. Bijloo.

DISCUSSION

Hoplotylus occupies an interesting but problematical taxonomic position. In common with the subfamily Hoplolaiminae Thorne, 1949, *Hop-*

lotylus has a massive stylet with well developed basal knobs placed closely together, a heavily sclerotized labial framework, and a glandular lobe which overlaps the intestine dorsally. However, *Hoplotylus* differs from all genera of the Hoplolaiminae in possessing but a single ovary and a tapering tail which in length exceeds the anal body width. The latter characteristic also distinguishes *Hoplotylus* from the subfamily *Rotylenchoidinae* Whitehead, 1958. *Hoplotylus* may conveniently be placed in the subfamily Pratylenchinae Thorne, 1949. This group contains at present five genera which show rather divergent characteristics, viz. *Pratylenchus* Filipjev, 1936, *Pratylenchoides* Winslow, 1958, *Radopholus* Thorne, 1949, and eventually *Rotylenchulus* Linford et Oliveira, 1940 and *Nacobbus* Thorne et Allen, 1944. The gravid females of *Hoplotylus, Pratylenchus, Pratylenchoides* and *Radopholus* are slender, whereas those of *Rotylenchulus* and *Nacobbus* are swollen. *Hoplotylus* differs from *Pratylenchoides* and *Radopholus* in possessing but a single ovary.

In having a single ovary and a posterior vulva *Hoplotylus* resembles *Pratylenchus*. It differs, however, from that genus by the longer glandular lobe of the esophagus, the posterior part of which overlaps the intestine dorsally over a long distance. In addition, *Hopotylus* has a high conoid lip region, a differently-shaped labial framework and a longer stylet.

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ZUSAMMENFASSUNG

Hoplotylus femina n.g., n.sp. wird beschrieben und abgebildet. Die wichtigsten taxonomischen Eigenschaften sind: das Kopfskelett stark chitinisiert, der stark entwickelte Mundstachel mit hervorragenden nach vorn zugespitzten gezähnten Knöpfen, der auffällig überschneidende Oesophagus, die unpaare, gestreckte, prävulvare weibliche Gonade, Vulva bei 87%, der konische, nicht auffallend kurze Schwanz und die fein geringelte Kutikula.

Die Gattung ist einstweilen am besten zu den Pratylenchinae Thorne, 1949 zu stellen. Diese Gruppe enthält bis jetzt Gattungen mit ziemlich auseinandergehenden Charakteren. *Hoplotylus* stimmt mit *Pratylenchus* in der unpaaren weiblichen Gonade überein, unterscheidet sich jedoch durch die Länge des Oesophagealdrüsenlappens, der dorsal aus dem Oesophagus austritt, und durch den längeren Stachel. Ausserdem hat *Hoplotylus* eine hohe konische Lippengegend.

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THE ATTRACTIVENESS OF ROOTS TO THE PLANT PARASITIC NEMATODES *MELOIDOGYNE JAVANICA* AND *M. HAPLA*

BY

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The attraction of various plants to phytoparasitic nematodes has been recognised for the past forty years. Apparently the first paper devoted to this subject was by LINDFORD (1939). He observed that the larvae of root-knot nematodes were attracted to the region of cell elongation just behind the root cap and that tissues rendered unattractive by cooking became attractive as they decomposed. He also observed that root-knot larvae congregated around anaerobic yeast colonies. Linford, although unable to explain these phenomena, came to the conclusion that root-knot nematode larvae did not respond selectively to the presence of an appropriate food supply but rather responded generally to stimuli emitted by non-food substances.

WIESER (1955) repeated Linford's work on a quantitative basis with excised tomato roots and found that the apical 2 mm of the root were repellent to nematodes whilst the next 6 mm were attractive. The portion behind this up to 16 mm was either neutral or slightly repellent to *M. hapla* larvae. WIESER (1956) repeated these experiments with bean, eggplant and soybean all of which are natural hosts to *M. hapla*. Rather surprisingly, he found that excised bean roots had a repellent effect on the larvae; excised roots of soybean and eggplant varied in their effect on the larvae, some being repellent, some attractive and some neutral. He attributed these results to an interplay between an attractive agent present in the living plant and a repellent associated with the chemical decay and breakdown of the root.

WALLACE (1958) suggested that for larvae of *Heterodera*, the me-

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chanism of attraction was by orientation to a concentration gradient of some chemical substance secreted by the plant roots.

Much work has been done on the effect of root diffusates on the hatching of *Heterodera* eggs but little or no work has been done on the attractant properties of these diffusates. This paper describes work on the physiological mechanisms associated with the growth of roots which cause them to be attractive to the larvae of phytoparasitic nematodes of the genus *Meloidogyne*.

MATERIALS AND METHODS

Materials

The nematodes used in these experiments were infective larvae of *Meloidogyne javanica* and *M. hapla* reared on tomatoes in pots of sterilized soil and originally obtained from egg sacs of females identified by examination of their perineal patterns. Egg sacs taken from the roots of these tomatoes were either left in 0.3M NaCl until required, when hatching was stimulated by placing them in distilled water, or were placed directly in distilled water. Freshly hatched nematodes were collected each day and were stored in petri dishes in shallow distilled water in a refrigerator. Only freshly hatched larvae (1-2 days old) were used.

Susceptible tomatoes of the variety Pan America were germinated on moist filter paper and grown in polythene containers, four plants per container (Brownell & Wood 1957), in complete nutrient solution (Arnon & Hoagland 1940).

Root Exudate

Root exudate was obtained by substituting glass distilled water for the nutrient solution, the roots first being rinsed thoroughly in distilled water. After 6-12 hours the roots were returned to the nutrient solution until further root exudate was required. The exudate in distilled water was concentrated *in vacuo* at low temperature with a rotary film evaporator. It was then stored in a refrigerated room at -10°C.

Chromatography

Paper chromatography was carried out in a constant temperature room at 25°C using butanol-acetic acid-water and saturated phenol-water for two-dimensional methods as previously described (Bird 1958) and ethyl acetate : pyridine : water (5 : 2 : 5) (Jermyn & Isherwood 1949) as a solvent for the uni-dimensional separation of sugars.

All the solvents were dried at room temperature overnight and phenol was removed by washing in ether. Amino acids were identified after dipping in 0.2% ninhydrin in acetone followed by heating for a few minutes at 105°C. Sugars were identified after spraying with a mixture of equal volumes of 0.15M aniline in butanol and 0.3M phosphoric acid in butanol (FRAHN & MILLS 1959) and also with benzidine reagent (benzidine 0.5 g, glacial acetic acid 10 ml, trichloracetic acid 10 ml, ethanol 10 ml).

Measurement of Attraction

The attraction to various substances was observed by using 1% "Difco bacto-agar" as the suspending medium; the agar was freshly

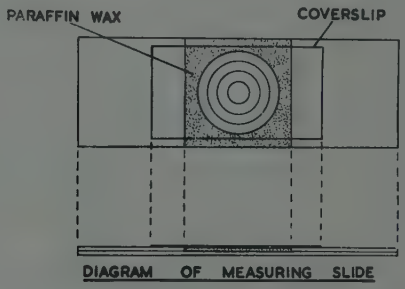

Figure 1. Diagram of measuring slide viewed from above and from the side.

prepared each day. Initial testing of attractant substances was done in small petri dishes. The test substance was dissolved in 5 ml distilled water and then 5 ml of 2% agar at a temperature of 44-46°C were added and the whole was rapidly stirred in an electric stirrer and then allowed to set. A core of this was obtained using a glass tube as a borer. The core was blown on to a clean glass slide and a 2 mm thick disc was cut from its centre and placed in the petri dish containing the nematodes suspended in 1% melted agar. The nematode-agar suspension was prepared by adding melted 2% agar at a temperature of approximately 45°C to an equal volume of the nematodes in distilled water; after shaking, the mixture was quickly transferred to the petri dish. This procedure prevented the formation of an interface between the disc and the rest of the agar.

Substances which were attractant under these conditions were tested quantitatively using slides which had four circles, each 2.5 mm apart,

etched on to them with hydrofluoric acid (Fig. 1). The etched circles were on the bottom of the measuring slide so that the larvae moved over a smooth surface and could not be trapped in grooves. The innermost circle was 5 mm in diameter and the outermost was 2 cm in diameter.

Using slides instead of petri dishes, a similar procedure, with smaller quantities, was adopted. The core was obtained by using a fine capillary tube as a borer, and the cut disc was transferred to the centre of the innermost ring. A coverslip was placed in position before the agar had set, air bubbles being excluded. The total number of larvae on the slide was counted. The easiest way to do this was to count the numbers between each set of rings and to total them. The number of larvae in the innermost ring was counted every ten minutes and the percentage number of larvae in this ring was plotted against time.

Tomato root tips, 5 mm in length, were placed in the inner circle and *E. coli* colonies were introduced in 1% agar discs after having been transferred from nutrient to 1% agar several hours beforehand.

Measurement of Redox Potential

Oxidation-reduction potentials were measured by the direct potentiometric method using a Jones pH meter. A Jones redox platinum electrode was inserted into the agar, either close to or within the disc. A salt bridge (2% agar saturated with KCl in a U-tube of glass of internal bore of 3 mm) connected this to the standard half cell — a calomel electrode in saturated KCl. Before and after use the electrodes were standardized at 38°C using a quinhydrone electrode. Differences between the observed and theoretical figures were never more than 5 mV.

RESULTS

Substances from Tomato root exudates

Chromatographic analysis of tomato root exudates collected from fully grown tomatoes for periods of 6-12 hours showed the presence of at least seven amino acids. In order of magnitude of their response to the ninhydrin reaction they were glutamic acid, α-alanine, aspartic acid, serine, valine, an unknown, and phenyl-alanine. These results agree in general with those obtained by Rovira (1959) for the same plant under similar conditions of light intensity and high temperature for the first two weeks of growth. That is that glutamic acid, aspartic acid, α-alanine and serine were produced in greater quantities than other

amino acids. ROVIRA collected his exudates over the much longer period of two weeks and was able to demonstrate some fourteen amino acids although some only occurred as traces. He examined exudates from *Phalaris* and clover as well as tomato and came to the general conclusion that glutamic acid was abundant in tomato and *Phalaris* but not in clover where glycine was abundant; this amino acid in turn was not abundant in *Phalaris* and tomato. The above amino acids and also glycine, methionine, leucine and iso-leucine were tested for attractiveness at concentrations of 0.1M in 1% agar discs. Glutamic acid was the only one to which the larvae were attracted but the attraction was only mild and transitory.

Neither concentrated nor unconcentrated tomato root exudates attracted root-knot larvae. However, if root exudates of both wheat and tomato were placed in contact with ion exchange resins, the cationic exchange resin Dowex 50 remained unattractive while the anionic exchange resin Dowex 1 became quite attractive. A similar result was obtained if 0.1M glutamic acid was used in place of the root exudate and again if seeds were allowed to germinate on moist ion exchange resins. Only the anionic exchange resin was attractive to root-knot larvae. This attraction was not rapid but the larvae appeared to remain close to the resin after they came into contact with it.

Concentrated tomato-root exudate, containing a brown precipitate of unknown composition, was unattractive on anionic resin; it was slightly attractive on cationic resin.

No sugars were indentified from concentrated tomato root exudates after running chromatograms in ethyl acetate : pyridine : water (5 : 2 : 5) solvent and spraying with aniline-butanol-phosphoric acid reagent but a slight positive reaction was obtained with the benzidine reagent at the origin of a butanol-acetic run. Glucose, fructose, ribose, xylose, arabinose, galactose and sucrose were all tested in 1% agar discs, and in no case was there any significant attractiveness to root-knot larvae.

These results suggest that under normal conditions acidic substances such as glutamic acid play a part in maintaining larval contact with the surface layer of the living protoplasm of the root.

Some naturally occurring substances.

The following substances, which occur naturally in plants, were tested in 1% agar discs: — gibberellic acid 0.1% (Lights), β-indolylacetic acid 0.1M (B.D.H. laboratory reagent), which was dissolved by neutralizing the solution with 0.1M sodium hydroxide, digitoxin

0.01% (Wyeth), oubaine (Arnaud) and 0.001M tryptophane (B.D.H.). Gibberellic acid was the only substance from this group to which root-knot larvae were attracted in petri dishes but this attraction was not pronounced enough, when compared with control discs of 1% agar, to be measured quantitatively on slides.

Colchicine salicylate 0.02%, originally suspected of being strongly repellent, proved neither repellent nor attractive.

The effect of pH

The attractant properties of various buffer solutions made up in 1% agar, over a pH range of 3-10.6 using four different types of buffers, were examined. Hydrogen ion concentrations at either extreme were repellent. As root-knot nematodes were not attracted to any particular hydrogen ion concentration, pH must play a secondary role in the physiological processes associated with root attraction.

The effect of carbon dioxide

Concentrations between 1-20% carbon dioxide in bicarbonate buffers in 1% agar at pH 8.0 and 6.7 were tested for attraction. Carbon dioxide was bubbled into the molten 1% agar for several minutes before it set and the fall in pH was measured using indicators. The larvae were not attracted to any particular concentration of carbon dioxide. Some discs showed more larvae in them during the counting period than others but this did not fit into a pattern which could be correlated with carbon dioxide concentration. Because it was not possible to maintain the carbon dioxide in the agar in an atmosphere of carbon dioxide and because of the method of introducing the carbon dioxide into the agar, the exact concentrations were not precisely known. In no case however, was there any significant attraction.

Redox potential

Infective larvae of *Meloidogyne javanica* and *M. hapla* were strongly attracted to some reducing agents. The most noticeably attractive chemical tested in these experiments was sodium dithionite (B.D.H.). In 1% agar it was attractive over a wide range of concentrations from 0.001M to 0.5M. 1M sodium dithionite was toxic to the larvae; 0.1M and 0.5M sodium dithionite were very attractive and slightly toxic so that in petri dishes a ring of dead larvae lay around the disc after twenty-four hours. At concentrations between 0.001M and 0.1M sodium dithionite the larvae showed an attraction similar to that found in various roots but it was more pronounced.

The movement of larvae towards and away from a 1% agar disc containing 0.1M sodium dithionite was measured in terms of redox potential. Two petri dishes containing similar numbers of larvae were used. In one the potential close to, but not in, the disc was measured at regular intervals and in the other the movement of larvae into and out of the disc was observed under a microscope at similar intervals. As the sodium dithionite diffused out of the disc the potential of the agar close to the disc dropped. This drop corresponded with an increase in the number of larvae coming into the disc. After an hour and a half, the potential beside the disc had ceased to drop. At this stage, a maximum number of larvae was counted in the disc. After two hours, the potential in the agar close to the disc had risen very slightly and the number of larvae in the disc was a third of what it had been thirty minutes before. Thus the larvae congregated in the area of lowest potential and remained there until the potential rose to that of the surrounding medium.

Other reducing substances, such as cysteine, glutathione and ascorbic acid, were tested at various concentrations. All these substances acted as mild attractants. Cysteine and ascorbic acid in 1% agar discs were more attractive than glutathione. Lower concentrations of cysteine, e.g. 0.001M, were more attractive than higher ones, e.g. 0.005M. These reducing agents were no more attractive than gibberellic acid and their attractant properties were not strong enough to measure quantitatively.

Cysteine was the only one which lowered the redox potential in agar significantly. Thus, the attraction of root-knot larvae to reducing substances appears to be governed, amongst other things, by the chemical nature of the substance itself.

The responses of root-knot larvae to two other substances were tested, viz., 0.01M riboflavine and 0.1% sodium thioglycollate. Neither of these were attractive.

Oxygen

Because these nematodes are aerobic organisms it was decided to see if they were attracted to areas of higher oxygen concentration.

Oxygen tensions in agar were not measured in these experiments. Larvae were attracted to an unoxygenated disc of 1% agar containing 0.05M sodium dithionite from a surrounding medium of 1% agar which had been bubbled with oxygen for several minutes before it set. On the other hand, the larvae in a surrounding unoxygenated medium of 1% agar were not attracted to an oxygenated disc of 1% agar. Thus,

while these creatures are undoubtedly aerobic, they are not attracted to a greater concentration of oxygen and will go to areas of lower oxygen tension in response to lower redox potentials.

Living systems

From the above experiments, it became apparent that the physiology of root-knot larval attraction was complex, involving various factors.

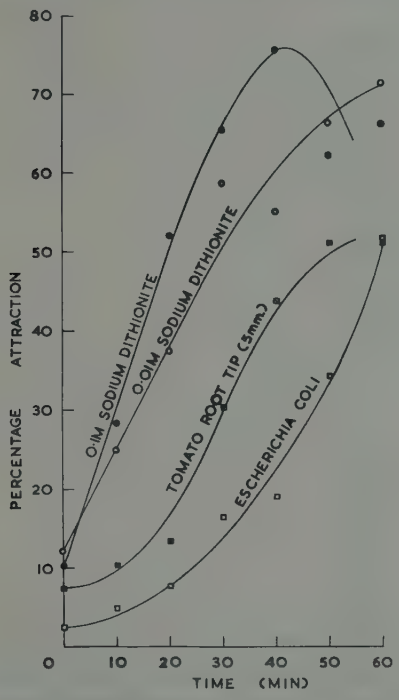

Figure 2. Graph showing relative attractive properties of sodium dithionite, tomato root and *E. coli* to similarly aged *M. hapla* larvae.

The principle factor appears to be movement along a gradient of decreasing potential. In order to keep the nematodes at the site of attraction, this gradient must be maintained and consequently, it was decided to test the effect of living systems in maintaining attraction and to attempt to correlate this with lowered oxidation-reduction potentials.

A natural attractant, the tomato root, was examined first. The redox

potential in the area of maximum attraction (the region of cell elongation) was, on an average, some 15 mV less than the potential 2 cm from the tip, so that a gradient was maintained along the root and also in the agar surrounding the root.

The aerobic bacterium, *E. coli*, is capable of rapidly lowering redox potential and, if fact, larvae of root-knot nematodes were attracted to agar discs containing these bacilli; again they moved along a potential gradient.

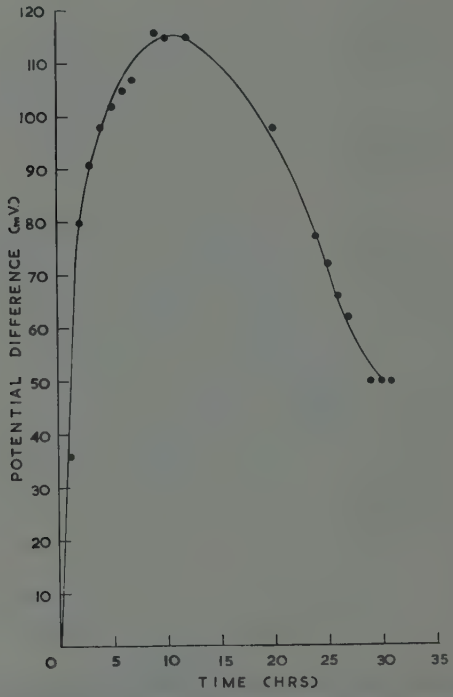

Figure 3. Graph showing the effect that 50,000 *M. javanica* larvae have on the redox potential of 5 ml of 1% agar.

The responses of *M. hapla* larvae to 1% agar discs of 0.1M sodium dihionite, 0.01M sodium dithionite, 5 mm of tomato root tip and a colony of *E. coli*, using the circled slides previously described, are shown (Fig. 2).

All the larvae used in this particular experiment were freshly hatched and were the same age. It is probable that different batches of dif-

ferently aged larvae would react at different speeds to attractive stimuli.

It can be seen that both concentrations of sodium dithionite are more attractive than either tomato root tip or *E. coli*. However, the living systems are capable of maintaining a gradient and so retain their attraction for longer periods. In the case of 0.1M sodium dithionite, attraction reached a peak after forty minutes. After this the number of larvae in the inner circle diminished as the concentration of sodium dithionite in the surrounding medium built up due to diffusion.

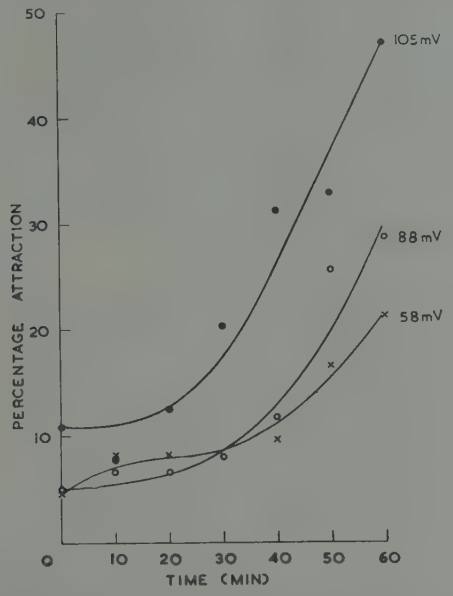

Figure 4. Graph showing relative attractive properties of 1% agar at three different oxidation-reduction potentials. These potential differences were caused by 50,000 *M. javanica* larvae in 5 ml of 1% agar. The 88 mV sample was taken three hours after the start of the experiment. The 105 mV sample six hours after the start and the 58 mV sample twenty eight hours after the start of he experiment.

During experiments which involved large numbers of larvae in agar in petri dishes, it was observed that, after a few hours, the larvae came together in clumps. After twenty-four hours the larvae had redistributed themselves in the agar and there was no longer any clumping. Preliminary tests indicated that the agar in which numbers of larvae had been moving about, acquired attractant properties. This phenomenon was tested in order to see if the attraction could be related to redox potential. Two tubes, each containing 5 ml of 1% agar and 50,000 larvae of *M.*

javanica were used and maintained under exactly similar conditions. The redox potential of one tube was measured over about thirty hours at regular intervals (Fig. 3) and the attractiveness of agar discs taken from the second tube was measured at intervals (Fig. 4). The redox potential (Fig. 3) dropped rapidly and reached its lowest point 10-12 hours after the start of the experiment. After this it started to rise again, or, in terms of Fig. 3, the difference in redox potential between the time of measurement and the start of the experiment decreased. Measurement of redox potential several days later showed that the potential difference continued to decrease to zero point, when the redox potential started to increase. Two agar samples were measured for attraction before the peak (Fig. 3) was reached and one was taken later in the experiment as the potential difference was decreasing. These results (Fig. 4) were interesting because they showed that maximum attraction was associated with the lowest potential or, in other words, the greatest negative redox potential difference. The 58 mV sample was taken twenty-five hours after the 88 mV sample and twenty-two hours after the 105 mV sample. If larval attraction had been due to a substance secreted by the larvae then this, the 58 mV sample, should have been the most attractive; instead it was the least attractive. Similar numbers of larvae of the same age were used in this experiment and they affected the potential in a similar manner. After the last core had been taken from the second tube its redox potential was measured and found to differ from the first tube by only 2 mV.

A large number of larvae (70,000) were placed in a small piece of dialysis tubing together with a little distilled water. This tubing was placed in 1% agar in a petri dish containing larvae. These larvae were not attracted to the sides of the dialysis membrane. Potential measurements at the side of the dialysis tube, at the edge of the petri dish and halfway between these two showed that the highest redox potentials were found close to the side of the dialysis tubing and the lowest at the edge of the petri dish, where the nematodes mostly congregated, while intermediate potentials were found in between. In this case, there was a gradient of increasing potential from the edge of the petri dish to the dialysis tubing.

The hypothesis is put forward that parasites of the genus *Meloidogyne* are primarily attracted to roots along a potential gradient caused by lower redox potentials. Contact with the root is maintained by these lower potentials and also by the acidic nature of the surface protoplasm (Lundegardh 1942). Factors such as root secretions, soil moisture

content (Wallace 1958), temperature and pH all influence this primary effect and play important secondary roles.

DISCUSSION

Differences in potential between roots and soil and along the root itself were measured by Lundegardh (1940 and 1942). He put forward a theory that the electro-chemical properties of the surface of living roots control the absorption of nutrient ions. Lundegardh (1942), working with cereals, found an average negative potential-difference between the soil and root of 62.7 mV when using moderate concentration of fertilizer. Lundegardh (1940) also measured the charge along a single root and found that this was not uniform. A maximum negative charge occurred in the region of cell elongation. Potential differences of 10-20 mV were observed between this part of the root and the areas above and below it.

Similar results were obtained in these experiments both for tomato and wheat roots.

Lundegardh (1942) referred to the surface layer of the root's protoplasm as the Z layer and stated that it might be characterized as an amphoteric colloid with predominantly acidic properties. This description of the nature of the surface layer of the root's protoplasm provides an explanation for the attraction of root-knot larvae to both root exudate and glutamic acid on anionic exchange resins.

The effect of lowered redox potentials on the physiology of nematodes was first studied by Rogers & Sommerville (1957) who found that stimulation of exsheathment of third stage trichostrongyle larvae in rumen fluid was more effective at low oxidation-reduction potentials. This work was extended to a study of the physiology of egg hatching in *Ascaris lumbricoides* (Rogers 1958). Here it was discovered that dissolved gaseous carbon dioxide at low redox potentials stimulated infective eggs to produce hatching fluid. The reducing agents used in this investigation, in order of activity, were sodium dithionite, cysteine and ascorbic acid. Optimal activity was obtained for all of these substances at concentrations of 0.02-0.04M.

The observations described in this paper make it possible to put forward explanations for some of Linford's (1939) results. For instance, attraction of root-knot larvae to rotting tissue may be explained as movement towards an area of lower potential caused by rapidly multiplying bacteria. Similarly, attraction of larvae to anaerobic colonies of yeast on agar could be explained as movement towards lower

potentials because yeasts will lower the redox potential of a culture (Hewitt 1950) and will do it more rapidly under anaerobic than aerobic conditions.

The attraction of members of the genus *Meloidogyne* to plant roots is probably dependent on other factors in addition to redox potential. Root exudations, and the nature of the surface protoplasm undoubtedly play a major role in maintaining the larvae close to the root, and in inducing their subsequent penetration.

I wish to thank Mr. F. Penman and Professor W. P. Rogers for their helpful criticism, Professor Rogers for allowing me to use the facilities of his Department, Professor J. G. Wood for allowing me to use his Department's glasshouse, and Dr. P. F. Brownell of the Botany Department for advice on the growing of plants in nutrient solution. Mr. P. P. Thomson assisted in all phases of the work, and his help is gratefully acknowledged.

ZUSAMMENFASSUNG

Das Anlockungsvermögen der Wurzeln auf die pflanzenparasitären Nematoden Meloidogyne javanica *und* M. hapla

Larven von *Meloidogyne javanica* und *M. hapla* werden hauptsächlich durch die der Zellstreckung unterworfenen Abschnitte in den Wurzeln der Wirtspflanzen angelockt. Dies ist die Region des niedrigsten Oxydations-Reduktions-Potentials. Es wurde die Hypothese aufgestellt, dass pflanzenparasitäre Nematoden hauptsächlich von den Wurzeln angelockt werden gemäss einem Potential-Gradienten, der durch Erniedrigung der Redoxpotentiale entsteht. Der Kontakt mit der Wurzel wird durch diese niedrigen Potentiale und auch durch die Säurenatur der Protoplasma-Grenzschichten herbeigeführt. Faktoren, wie Wurzelsekrete, Bodenfeuchtigkeitsgehalt, Temperatur und pH, beeinflussen sämtlich diesen primären Effekt und spielen auch sekundär eine wichtige Rolle.

Eine chromatographische Analyse zeigt, dass Glutaminsäure die häufigste Aminosäure in Tomatenwurzelexudaten ist. Larven werden durch sie in 1% Agar angelockt. Die Anlockung wird bei Verwendung eines Anionen-Austauschers verstärkt.

Reduzierende Stoffe, wie Natriumdithionit, Cystein und Ascorbinsäure, wirken auf die Wurzelgallenlarven anlockend, namentlich Dithionit. Giberellinsäure lockt die Larven ebenfalls an, während β-Indolylessigsäure, Trytophan, Dignitonin, Ouabain, Riboflavin und Natriumthioglycollat unwirksam sind.

Lebende Systeme sind fähig, die Existenz eines Gradienten aufrecht zu erhalten und damit auch ihre Wirkung für längere Zeit beizubehalten. Wurzeln, *E. coli* und die Larven selbst können sämtlich das Potential erniedrigen, zur Entstehung eines Gradienten führen und damit eine Anlock-Reaktion verursachen.

Von der obigen Hypothese wurde Gebrauch gemacht, um eine Erklärung für die interessanten, aber nicht vollständig klaren Ergebnisse vorzubringen, die von einem früheren Forscher auf diesem Gebiet erhalten wurden.

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EXPERIMENTS ON THE INTERACTION OF *HOPLOLAIMUS UNIFORMIS* AND *FUSARIUM OXYSPORUM F. PISI* RACE 3 AND ITS IMPORTANCE IN "EARLY YELLOWING" OF PEAS

BY

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The disease of peas, which is now called "early yellowing" (Dutch "vroege vergeling") was first mentioned by SCHREUDER (1951), who wrongly identified it with "St John's disease" of earlier authors. The Dutch name of the disease is derived from the symptoms in the overground parts of the plants: a yellowing appears early in the season in the lower leaves and spreads slowly to those higher up the stem. The yellowing starts at the edges of the leaflets and progresses inwards. Withering and shrivelling may soon follow the yellowing and occasionally under greenhouse conditions the leaves become very thin and flaccid before the yellowing is conspicuous. Plants with the symptoms described above are always seriously affected by root rot but not all plants with root rot in an affected field show overground symptoms. Favourable weather conditions in May and June seem to repress the development of the latter. Figure 1 shows the relation between the occurrence of root rot and overground symptoms in a field experiment. The root rot is considered the primary and most important symptom of the disease. The cortex of the diseased roots is blackened and necrotic. Thin lateral roots are often completely dead and break off easily. The vascular bundle of affected roots shows an orange discolouration, which often starts as more intensely coloured spots at points where damaged lateral roots are attached to thicker roots. Where the attack is especially severe even the vascular bundle in the stem may be orange. In the summary of the article by SCHREUDER (1951) this disease is wrongly called a pea *wilt* disease. Neither in the article nor in the Dutch summary is it described as such. Actually affected plants remain more rigid the more severely they are stunted. Unlike *Fusarium* wilt and "St John disease" (*Heterodera goettingiana*) "early yellowing" may affect peas

as early as the end of April and at low soil and air temperatures (hence the name). Such an early attack may stunt the plants severely. However, only rarely do they die before flowering. Usually they produce one or two small pods.

According to SCHREUDER (1951) this disease is closely associated with the occurrence in the roots of the diseased plants of a strain of

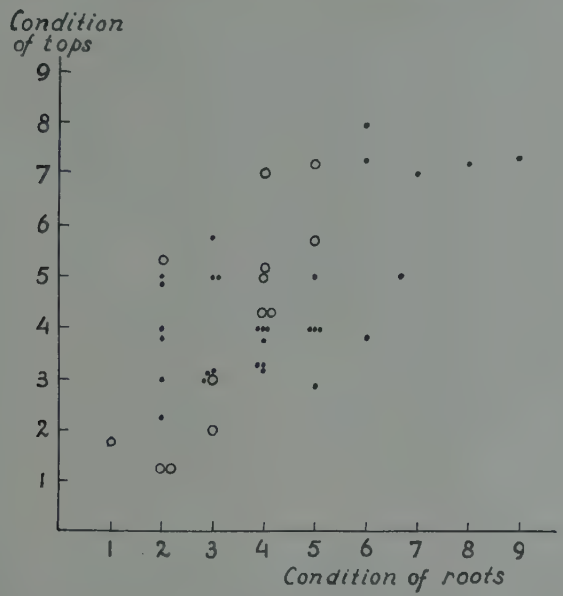

Fig. 1. Relation between the occurrence of root rot and symptoms of "early yellowing" in the overground parts of peas in a field experiment. Horizontal: degree of root rot; vertical: symptoms in overground parts. 1 = serious root rot and serious overground symptoms; 9 = almost healthy roots or tops; . on untreated soil and after different treatments with "Mylone". o after DD treatment (three dosages).

the fungus *Fusarium oxysporum* SCHL. em. SN. & *H. f. pisi* LINF. She classified it as race 3 of this fungus, as its pathogenic characters were markedly different from those of the races 1 and 2.

However, no symptoms in either roots or overground parts could ever be obtained by growing peas in autoclaved soil, which had been inoculated with *F. oxysporum* isolated from plants suffering from "early yellowing". A very limited rot developed in pea roots, which

had been damaged by cutting them before bringing the plants in contact with the fungus (LABRUYÈRE & SEINHORST 1954).

With only one exception (after a DD treatment, see p. 342), fields, where the disease was found, contained medium to high populations of *Hoplolaimus uniformis* THORNE (300-3000 nematodes per 500 g of soil). It was never seen in fields on soil types which are known to be practically free from this nematode such as sandy loam and clay soils (LABRUYÈRE & SEINHORST, 1954). When nematodes were removed from soil, on which "early yellowing" occurred, either by mechanical means (elutriation) or by treatment with Mylone [1]), peas grown on it in the field or in pots were not or only lightly attacked. However, peas grown in this treated soil after reinoculation with *Hoplolaimus uniformis* were severely attacked again (Table I, SEINHORST, 1954). Apparently there is a relation between medium to high population densities of *H. uniformis* in the soil and "early yellowing". The symptoms of the disease are also much more similar to those of a nematode disease than to those of attacks by *F. oxysporum*. However, "early yellowing" does not occur on all soils with high population densities of *H. uniformis*. Peas were healthy in a field at Rockanje (pop. density 300-650 nematodes per 500 g), in pot experiments with natural soil from different localities containing 100-3000 nematodes per 500 g, and in sand inoculated with large numbers of *H. uniformis*. In the last the nematodes were seen feeding on the roots in minute cracks in the cortex. In gardens at Ede and Bennekom and in several fields at Hoeven, "early yellowing" of the overground parts of peas occurred on an area much smaller than where medium to high population densities of *H. uniformis* were found. Some root rot occurred in healthy looking plants from both parts of the field. In one field the degree of attack was more severe where peas had been grown in the previous year than where they had not, although there was no material difference in eelworm population levels. The results of inoculation experiments with *H. uniformis* from fields on which peas had never been grown, or not grown for many years, do not suggest major differences in the capacity of different populations of this nematode to attack peas. Apparently "early yellowing" of peas is caused by the interaction of *H. uniformis* with one or more other factors. Such a factor could be *Fusarium oxysporum f. pisi* race 3 of SCHREUDER (1951), a fungus, isolated from almost all pea plants investigated which showed the typical symptoms of the disease.

1) Active ingredient 3-5 dimethyl tetra hydro-1-3-5-2H-thiadiazine-2-thione.

TABLE I

Results of inoculation experiments on "early yellowing" of peas var. Unica. (1), 3), 4), and 6) after SEINHORST, 1954)

Treatment of soil	No. of pots	Population densities of *Hoplolaimus uniformis* per 500 g of soil		Number of peaplants affected by "early yellowing"			Means of ratings per plant for size and disease
		beginning of experiment	end of experiment	severely attacked	lightly attacked	healthy	symptoms
1) Soil Ede	2	appr. 2000	?	6 } 11	0 } 3	0 } 1	4 (group rating)
2) Soil Hoeven	3	„ 1600	2200	5 }	3 }	1 }	5[1])
3) As 1); nematodes removed mechanically	2	„ 50?		0	0	6	8 (group rating)
4) 3) + *Tylenchorhynchus* sp. and *Pratylenchus* sp.	2	„ 50?	8	0 } 1	0 } 2	6 } 24	8 (group rating)
5) 2) treated with Mylone	4	„ 22	22	1	2	12	7.7
6) 3) + *H. uniformis* from Ede	2	„ 2000	700	6	0	0	4 (group rating)
7) 5) + *H. uniformis* from Hoeven	2	„ 1500 in top 10 cm of pots	880	3 } 14	3 } 3	2 } 4	4.5
8) 5) + *H. uniformis* from Rockanje	2	the same	580	5	0	2	3

1) 1 severely stunted, only top leaves not withered 7, 8, 9 healthy, but different sizes.

INOCULATION EXPERIMENTS ON FOAM AGAR PLATES

As the root rot described above is considered the primary symptom of "early yellowing", attempts were made to reproduce this symptom experimentally. The "foam agar plate" technique (DEN OUDEN, 1958) was used because

1. Activities of the organisms and the development of symptoms could be followed from day to day.
2. Separate roots could be inoculated individually. As pea varieties are very homogeneous each inoculation can be considered as a replicate, so reducing the number of plants and the number of nematodes necessary in the experiments. As the latter had to be picked by hand from a mixture extracted from soil this is a great advantage.
3. Experiments cannot be done in partly sterilized soil as often *Hoplolaimus uniformis* does not long survive in it.

A drawback of the method was that the experiments had to be finished before the agar began to dry out. However, pea plants could be kept growing for more than four weeks at 15° to 18° C which was sufficient for the development of symptoms in the roots.

Three experiments were made with single pea plants (variety Unica) in foam agar cultures. The same treatments were used throughout: —

1. *Hoplolaimus uniformis* five to seven inoculations per bag with 50-100 specimens placed near to roots.
2. *Fusarium oxysporum* five to seven inoculations per bag with mycelium.
3. Inoculation with both organisms as in (1) and (2).
4. Controls, not inoculated.

In the first experiment, one plant was used per treatment, in the second four and in the third one. Altogether there were six plant replicates for each treatment. The results of the second experiment are given in Table II.

The plants inoculated with *H. uniformis* only developed a slightly grey discolouration of a limited length of root. Some inoculations with *Fusarium oxysporum* resulted in discolouration and superficial destruction of the root cortex near the inoculation sites. This was especially so where the inoculum had been placed in contact with the roots. Where it had been put a short distance from the roots as in the third experiment

Fig. 2. Root system of pea plant four weeks after inoculation with *Fusarium oxysporum*.

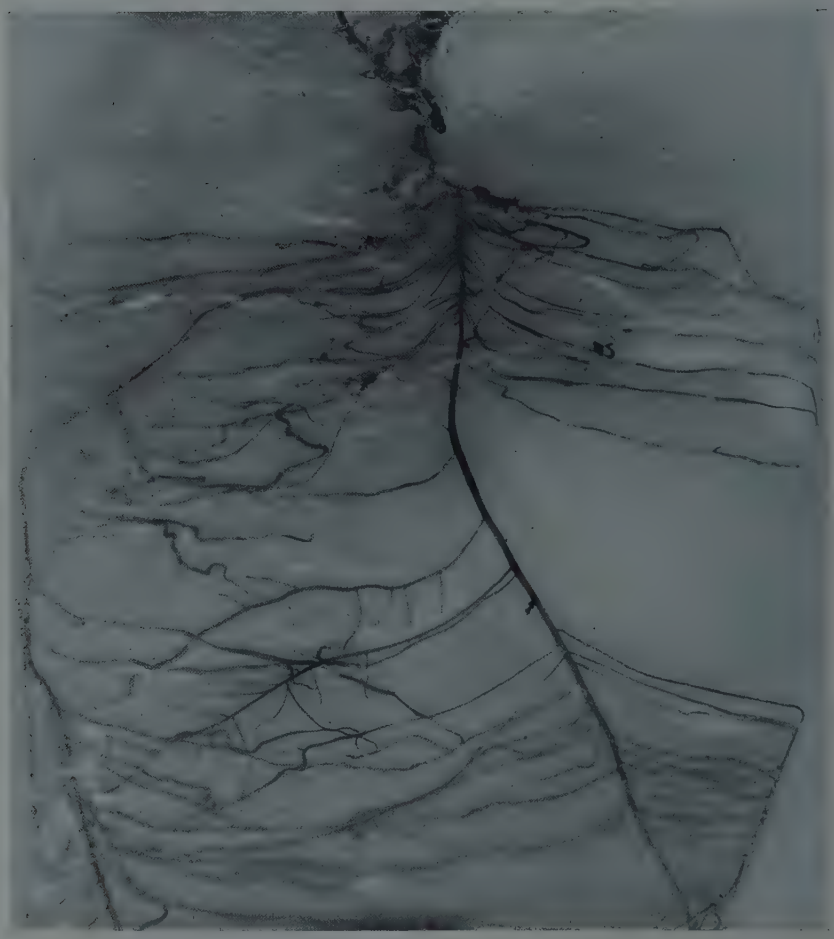

Fig. 3. Root system of pea plant four weeks after inoculation with *Hoplolaimus uniformis* and *Fusarium oxysporum*.

Fig. 4. Section of a root three weeks after inoculation with *Hoplolaimus uniformis* and *Fusarium oxysporum*.

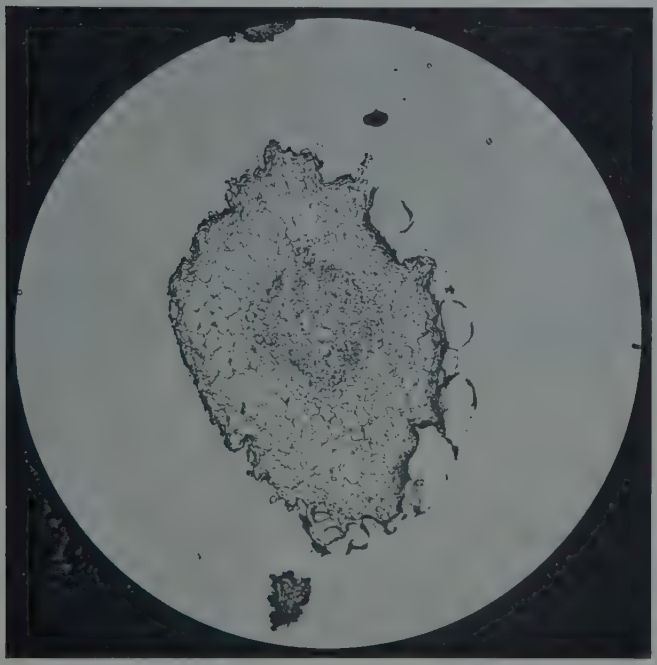

Fig. 5. Section of a root three weeks after inoculation with *Fusarium oxysporum*.

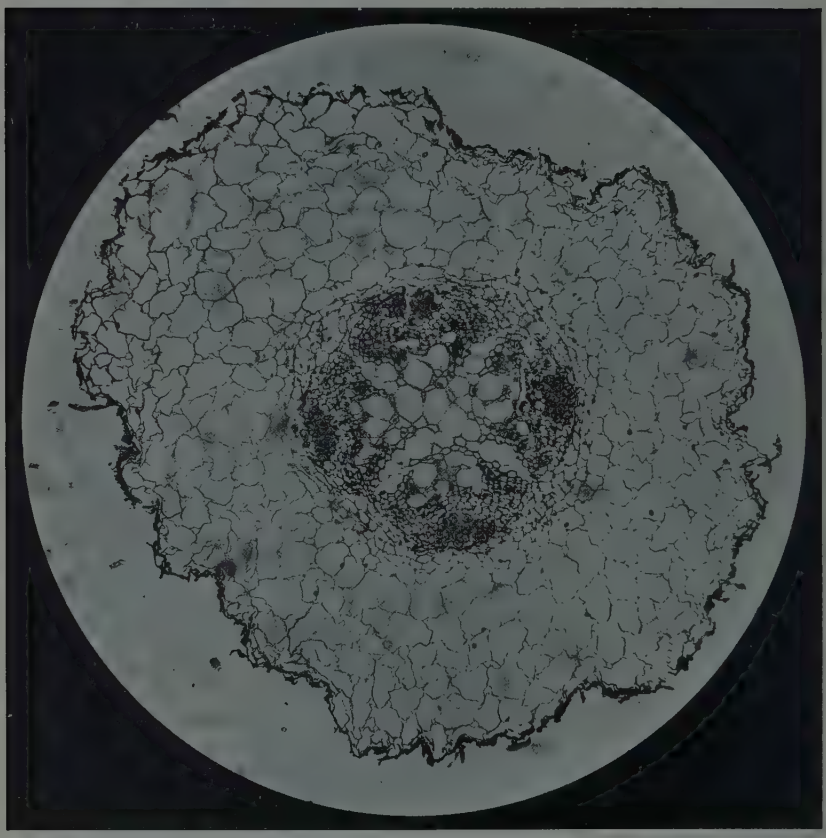

Fig. 6. Section of a root three weeks after inoculation with *Hoplolaimus uniformis*.

the roots did not suffer visible damage although the mycelium grew through the agar and on the root surface (fig. 2).

However, most inoculations with both organisms resulted in dark brown discolouration often followed by complete decay of the root cortex near the inoculation site (fig. 3). Measurements of the length of discoloured root and counts of the number of inoculations resulting in decay of the cortex in the second experiment are shown in Table II. Three weeks after inoculation eight (out of twenty four) inoculations with *F. oxysporum* and twenty three (out of twenty eight inoculations)

TABLE II

Results of an inoculation experiment on foam agar plates with H. uniformis *and* F. oxysporum forma pisi *race 3*

Inoculated with:	Plant No.	Number of inoculations per plant	Two weeks after inoculation: Length of discolored root per inoculation in mm.	Two weeks after inoculation: Condition of discoloured parts of roots	Three weeks after inoculation: Number of inoculations which resulted in: decay of cortex	Three weeks after inoculation: Number of inoculations which resulted in: no decay of cortex
A Not inoculated	1	—	0	—		
	2	—	0	—	—	—
	3	—	0	—		
	4	—	0	—		
B *H. uniformis*	5	4	1	slightly grey		
	6	5	4	slightly grey	0	0
	7	5	1	slightly grey		
	8	4	0	slightly grey		
C. *F. oxysporum*	9	7	5	light brown	8[1])	16[3])
	10	6	3	„ „	(12 mm	
	11	7	1	„ „	per inocu-	
	12	4	6	very light	lation)	
D *H. uniformis* +	13	7	4	brown to	23[2])	5[3])
F. oxysporum	14	7	8	black	(16 mm	
	15	7	8	„	per inocu-	
	16	7	11	„	lation)	

1) Severest case as fig. 5.
2) Lightest case as fig. 5 more generally as fig. 4.
3) Difference between C and D highly significant ($P < 0.01$).

with *H. uniformis* and *F. oxysporum* had resulted in cortex decay. The mean length of affected root per inoculation in the latter was three and a half times that in the former. Three weeks after inoculation sections were made of attacked portions of roots from each treatment.

Inoculation with *H. uniformis* produced only cracks in the cortex (fig. 6). Eelworms had been seen active in these cracks. Inoculation with *F. oxysporum* caused destruction of the outer layer of the cortex in some cases (fig. 5) but this destruction never reached the vascular bundle. However, inoculation with both *F. oxysporum* and *H. uniformis* often resulted in complete destruction of the cortex (fig. 4). The worst attack by *F. oxysporum* was comparable to the lightest seen after inoculation with both *F. oxysporum* and *H. uniformis*. Therefore the ratio between severity of attack after inoculation with both organisms and that after inoculation with *F. oxysporum* only was much greater than the 3.5 to 1 mentioned above.

CONCLUSIONS

The experiments confirm the conclusion already drawn from other observations that neither *Fusarium oxysporum* SCHL. em. EN & *H. f. pisi* LINF. race 3 of SCHREUDER (1951) nor *Hoplolaimus uniformis* THORNE can cause serious root rot in peas when they operate separately. However, interaction of both organisms can lead to extensive decay of the root cortex which is the chief symptom in the roots of peas suffering from "early yellowing".

"EARLY YELLOWING" AFTER DD TREATMENT OF THE SOIL

One case of typical "early yellowing" was found on soil which was practically free from nematodes. In a field experiment (SEINHORST, BIJLOO & KLINKENBERG, 1956) peas were not only severely attacked by early yellowing on the untreated plots but also on those treated with DD seven months before sowing. It was unlikely that residual toxicity of DD in the soil alone had caused this as the symptoms were exactly the same as those on the untreated plots and did not appear until six weeks after sowing. Possibly traces of DD in the soil made the pea roots susceptible to attack by *Fusarium oxysporum f. pisi* race 3. Since the fungus was unable to attack pea roots in sterilized media, it is unlikely that the changed soil conditions after DD treatment would enable it to injure the peas directly. Also, since peas grown in the same soil after harvest remained completely healthy (eight pots, thirty plants), there did not appear to have been a change in pathogenicity.

The author wish to thank Miss A. Kits and Miss G. Engels for assisting in inoculating the plants and making microtome sections.

ZUSAMMENFASSUNG

Untersuchungen über die Wechselwirkung von Hoplolaimus uniformis *und* Fusarium oxysporum f. pisi *Rasse 3 und ihre Wichtigkeit zur frühen Vergilbung von Erbsen.*

In einigen Versuchen nach der Schaumagarplattenmethode von Den Ouden wird nachgewiesen, dass durch *Hoplolaimus uniformis* Thorne oder *Fusarium oxysporum* forma *pisi* Rasse 3 keine ernsthaften Krankheitssymptome an den Wurzeln von Erbsen hervorgerufen werden. Durch den Angriff beider Organismen zusammen wurde jedoch die Wurzelrinde von Erbsen ganz vernichtet. Auch trat hierbei eine rötliche Verfärbung des Zentralzylinders der angegriffenen Wurzeln auf. Die unterstützt die Auffassung, dass frühe Vergilbung bei Erbsen, wobei man immer *H. uniformis* im Boden und *Fusarium oxysporum pisi* in den faulenden Wurzeln findet, durch Zusammenwirken der beiden Organismen verursacht wird.

Frühe Vergilbung trat auch auf nach Bodenentseuchung durch DD. Wahrscheinlich hat Schädigung durch dieses Nematizid die Erbsenwurzeln hier für *Fusarium* anfällig gemacht.

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THE INFLUENCE OF TEMPERATURE ON THE SURVIVAL OF SOME SPECIES OF THE GENUS *MELOIDOGYNE*, IN THE ABSENCE OF A HOST [1])

BY

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Environmental temperature is important to a cold-blooded animal such as a nematode because of its direct influence on the nematode's growth and activities. NIELSEN (1949) found that the oxygen consumption of *Mononchus papillatus* Bastian 1855, was increased by a factor of 5.5 when the temperature was increased from 55.4 to 86° F. The level of activity dictated by temperature can become critical to a soil nematode in the absence of a food source. More precise knowledge than is available concerning these temperature relationships would be of value in estimating the duration of nematode infestations, and would help in the development of cultural control measures.

Several authors have considered the temperature relationships of rootknot nematodes prior to their revision by CHITWOOD (1949). CUNNINGHAM (1936) stated that winter conditions on Long Island with soil temperatures of 22° F. did not destroy or noticeably reduce the root-knot nematode infestation of potato plants. BESSEY & BYARS (1915) and GODFREY (1923) support Cunningham's contention by reporting that root-knot nematodes are known to maintain themselves in such cold winter regions as northern Indiana, Michigan, and Nebraska. NEWHALL (1934) however, cited a case where badly infested peat soils were found free of infestation following a period when air temperatures were —15° F. for 24 hours. FICHT (1939) was unable to demonstrate that root-knot nematode was capable of surviving the winter in northern Indiana. NEAL (1889) thought that root-knot nematodes could not establish themselves in a region where the soil was

1) Based on a thesis submitted to the University of California for the degree of Doctor of Philosophy.

2) Formerly at University of California, Berkeley, California.

annually frozen at depths of 6 to 10 inches. These conflicting points of view indicated the need for additional work on this subject.

MATERIAL AND METHODS

Investigations on soil temperature influences on survival of root-knot nematodes were designed so that factors other than temperature were as near optimum as possible. Consequently, the soil used was a light sandy loam which had been sterilized to exclude possible nematode parasites, and soil moisture was maintained between 60-100% of moisture equivalent (here after abbreviated as M.E.) which in previous tests had proved to be the best moisture range.

Meloidogyne incognita acrita Chitwood, 1949, collected from southern California was the species mostly used, although *M. hapla* Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949 were included for comparison. The eggs and larvae were tested separately to determine differences, if any, in survival between the two stages. Egg inoculum was obtained by picking off egg masses from galled roots and placing them on strips of moist filter paper. Larval inoculum was obtained by placing egg masses on pieces of gauze supported by a copper screen in a petri dish containing enough water to wet the gauze but not submerge it. In 3 to 4 days at room temperature large numbers of larvae could be collected in the water below the screen. Larval or egg inoculum was thoroughly mixed into soil contained in pint jars which were then transferred to constant temperature cabinets. Jars were sealed with polyethylene which allowed carbon dioxide and oxygen exchange with the atmosphere, but prevented moisture loss in excess of 10% of the original moisture level.

After an exposure period at a specific temperature, the soil was transferred to 4 inch clay pots, placed in the greenhouse, and planted with tomatoes. Five weeks later the roots of the tomatoes were examined and any galls present were counted. Gall counts were equated to surviving larvae or eggs. It is probable that this method did not give an exact survival count, but it was felt that it gave a reliable relative indication of survival, and also indicated whether or not the surviving nematodes were infective.

Two types of controls were used, the first estimated the viability of the inoculum by introducing the indicator plant into the soil immediately after inoculation (= viability check in Tables I-IV). The term control was only used in those experiments where infested soil was exposed to an optimum survival temperature alongside exposure to other tem-

peratures. Because the optimum temperature was not known in some of the first experiments, only a viability check was used.

EXPERIMENTS AND RESULTS

Survival at various temperatures

Soil infested with eggs or larvae prepared in the manner described were exposed to temperatures of 32°, 40°, 50°, 60°, 70°, 80°, 90°, and 100° F. for different times. Most experiments were repeated 2 or 3 times to confirm previous results or to adjust the exposure periods as dictated by the initial tests. Figs. 1 and 2 represent the maximum survival

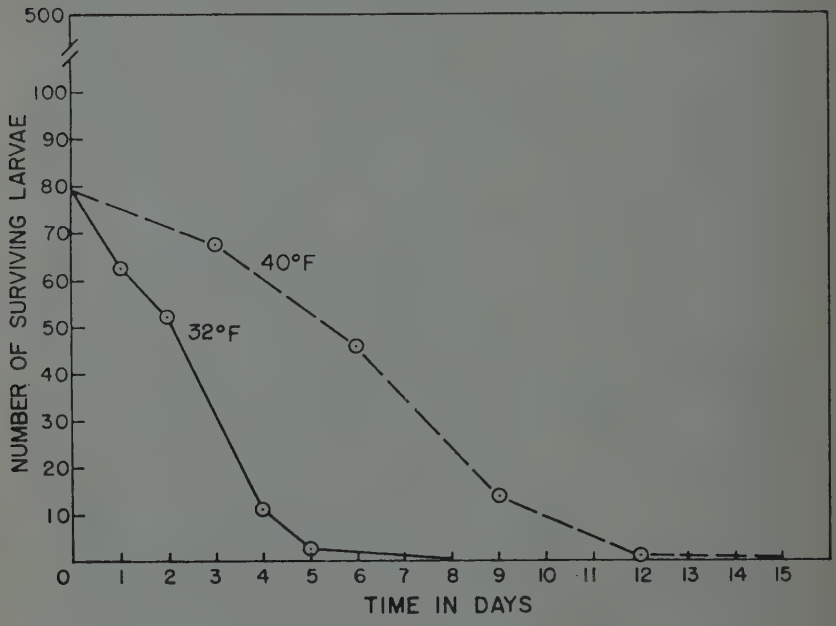

Fig. 1. The influence of temperature on the survival of larvae of *M. incognita acrita* in soil. Number of larvae determined by gall counts on an indicator plant.

of larvae attained in any series of experiments at a given temperature. Fig. 3 represents the survival of eggs at the temperatures indicated.

The effect of 32° and 40° F. on larvae was unexpected since soil temperatures are often this low for considerable periods in areas where root-knot nematodes are known to occur. If these laboratory

Fig. 2. The influence of temperature on the survival of larvae of *M. incognita acrita* in soil. Number of larvae determined by gall counts on an indicator plant.

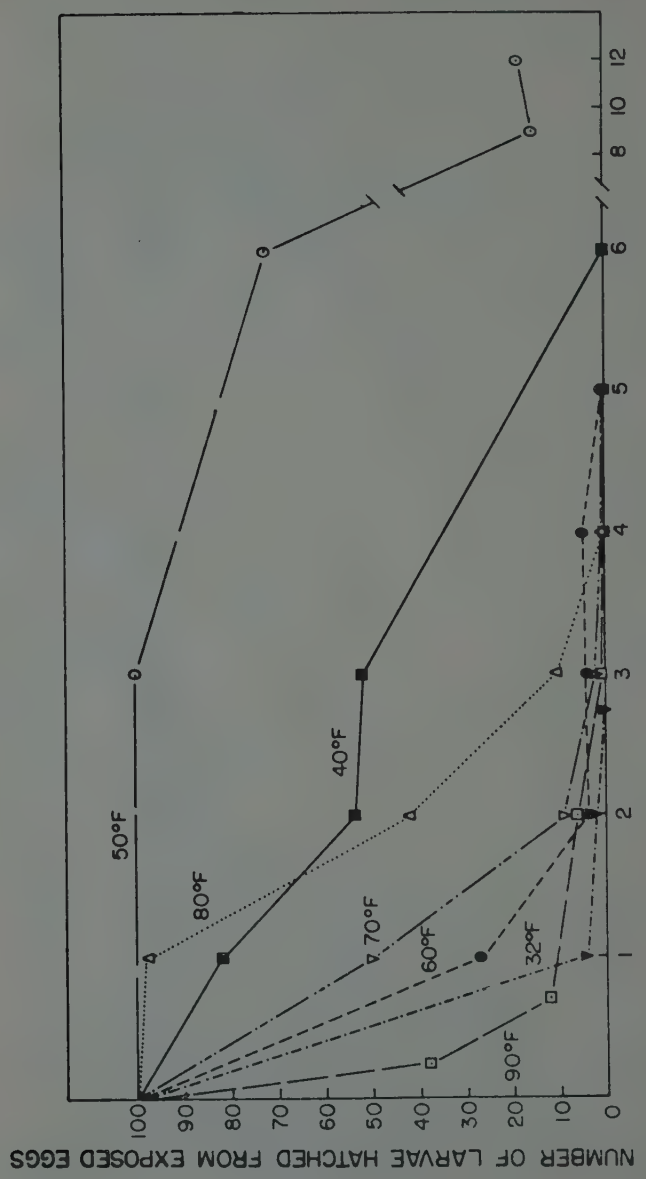

Fig. 3. The influence of temperature on the survival of eggs of *M. incognita acrita* in soil. Number of larvae determined by gall counts on an indicator plant.

results simulate field conditions, then it is possible that *M. incognita acrita* may overwinter in the egg stage in moderately cold regions. A second possibility is that under field conditions the soil temperature changes under the surface are so gradual that the larvae are able to adapt themselves to lower temperatures. A third is that resistance to cold varies with the different species of *Meloidogyne*. Investigations on the second and third possibilities are reported in the following sections.

Preconditioning experiment

Larvae of *M. incognita acrita* were exposed to successively lower temperatures to determine whether preconditioning could increase the length of survival at 32° F. The following comparisons were made: (1) larvae taken from room temperature and immediately placed at 32° F. for seven days, (2) larvae exposed for successive seven day periods at 50° and 32° F., and (3) larvae exposed for successive seven day periods at 50°, 40°, and 32° F. A series of jars taken from the 50° F. cabinet at three successive weekly intervals served as controls. The results presented in Table I indicate that no adaptation by larvae to lower temperature occurred.

TABLE I

Number of surviving larvae of Meloidogyne incognita acrita *(as indicated by galls on indicator plant) per pint of soil infested with 500 larvae and exposed to 32° F. following different preconditioning temperatures.*

Temp.	Exposure time, weeks	Temp.	Exposure time, weeks	Temp.	Exposure time, weeks	No. of root-knot galls on indicator plant
32 F.	1					0.0+
50 F.	1	32 F.	1			.5+
50 F.	1	40 F.	1	32 F.	1	.1+
50 F.	1					24.4*
50 F.	2					16.2*
50 F.	3					16.4*
Viability check					....	37.7*

LSD 5% — 6.8
LSD 1% — 9.1

\+ Mean of 10 replications
* Mean of 5 replications

Interspecific comparisons

There is nothing in the literature indicating differences in resistance to low temperatures among the species of *Meloidogyne*, although BROWN (1955) reported that in Great Britain only *M. hapla* seemed to persist in field infestations. The fact that *M. hapla* is referred to as the "Northern Root-knot Nematode" suggests the possibility that it is better adapted to colder climates. This possibility was tested by comparing larvae of *M. hapla* and *M. incognita acrita* at 32° and 40° F. The results of this comparison, presented in Table II, showed that *M. hapla* was better able to survive these temperatures.

TABLE II

Comparison of the number of surviving larvae (as indicated by galls on indicator plant) per pint of soil infested with 500 larvae of either M. hapla *or* M. incognita acrita, *and exposed to 32° F.*

Exposure Time	Temp.	Number of root-knot galls on indicator plants [a] *M. hapla*	*M. incognita acrita*
3 days	32°F.	11.0	15.8
7 „	„	6.6	0.0
10 „	„	7.2	0.0
13 „	„	1.0	0.0
16 „	„	.2	0.0
7 days	40°F.	8.8	0.2
14 „	„	5.4	0.0
21 „	„	7.4	0.0
28 „	„	7.6	0.0
Viability check		27.8	44.8

[a] Mean of 5 replications.

Additional experiments were conducted to provide comparisons among (1) the egg stages, (2) different geographical populations of *M. hapla*, and (3) a third species, *M. javanica*. The results, presented in Tables III and IV, indicated that larvae and eggs from both populations of *M. hapla* survived better at 32° F. than did these from *M. incognita acrita* and *M. javanica*. There also appeared to be a much greater interspecific difference in cold temperature resistance in the egg stage than in the larval stage. This difference in resistance to low temperatures among the three species tested of *Meloidogyne* is evidently one of the factors contributing to the confusion in the literature regarding the effect of low temperatures on root-knot nematodes.

Table III

A comparison of the number of surviving larvae (as indicated by galls on indicator plant) per pint of soil infested with 500 larvae of either M. incognita acrita, M. javanica, *or one of two populations of* M. hapla, *and exposed to 32° F.*

Exposure Time	Number of root-knot galls on indicator plants [a]	
	M. hapla (Davis)	*M. hapla (San Francisco)*
2 days	7.8	5.6
7 „	1.0	14.2
11 „	1.6	2.8
14 „	.6	0.0
Viability check	69.2	13.2
Exposure Time	*M. incognita acrita*	*M. javanica*
2 days	31.6	10.8
7 „	0.0	.8
11 „	0.0	0.0
14 „	0.0	0.0
Viability check	55.2	18.2

[a] Mean of 5 replications.

Table IV

A comparison of the number of surviving eggs (as indicated by galls on indicator plant) per pint of soil infested with 5 egg masses of either M. incognita acrita, M. javanica, *or one of two populations of* M. hapla *and exposed to a temperature of 32° F.*

Exposure Time	Number of root-knot galls on indicator plants [a]	
	M. hapla (Davis)	*M. hapla (San Francisco)*
25 days	69.2	44.6
50 „	46.6	51.2
90 „	51.6	34.0
Viability check	160.4	47.4
Exposure Time	*M. incognita acrita*	*M. javanica*
25 days	4.6	.5
50 „	2.6	.2
90 „	0.0	0.0
Viability check	68.4	59.0

[a] Mean of 5 replications.

The effect of temperature on the rate of egg hatching.

The above experiments indicated that eggs were more resistant than larvae to cold temperatures. Therefore, the chances of *Meloidogyne* species persisting when soil temperatures are low may depend on their stage of development. It is, therefore, important to know what factors influence hatching rates and the following experiment was designed to find the effect of temperature. Ten petri dishes with five egg masses each, on strips of moist filterpaper, were placed at each of the following temperatures: 40°, 50°, 60°, 70°, 80°, and 90° F. At intervals of two or three days the water in the bottom of the petri dishes was collected and replaced with water of the same temperature. Larvae in the collected water was counted. Results appearing in Fig. 4 showed that by the end

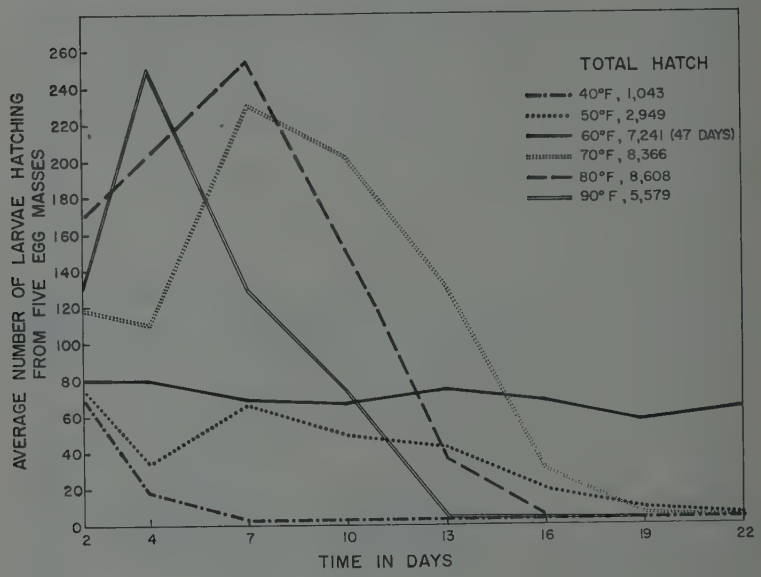

Fig. 4. The influence of temperature on the rate of egg hatching of *M. incognita acrita.*

of the 22nd day an appreciable hatch was occurring only at 60° F. At this time the unhatched eggs from each petri dish from the 50°, 70°, 80°, and 90° F. series were placed into pots containing soil and an indicator plant to determine the viability of the unhatched eggs. Galls on these plants averaged 26, 0.7, 1.5, and 36 respectively. This indicat-

ed that hatching at 50° and 90° F. was partially arrested, but continued when the eggs were removed to a more suitable temperature, whereas at 70° and 80° F. hatching had neared completion. The data in Fig. 4 also indicated that at temperatures above 60° F. hatching reaches a peak on the 4th to 7th day, but at 60° F. the hatching rate remains nearly constant. Eggs at 60° F. were allowed to remain at this temperature for 47 days and during the whole of this time an appreciable hatch was obtained. Unhatched eggs at the end of 22 days at 40° F. were transferred to 70° F. until completion of hatching. The 1,043 larvae that emerged prior to transfer to 70° F. probably represented larvae which had already emerged within the egg mass before exposure to 40° F. The total emerged larvae from eggs held at 40° and then transferred to 70° F. was only 60% of the hatch that occurred in the series which remained at 70° F., thus indicating that considerable mortality occurred during the initial exposure of 22 days at 40° F.

DISCUSSION AND CONCLUSIONS

For *M. incognita acrita,* 50° F. most closely approached the optimum temperature for survival of eggs and larvae which at this temperature survived for more than a year. Between 50° and 60° F. the activity of the larvae and their utilization of food reserves presumably increases so that their life span in the absence of food is decreased by at least 50%. This compares with the 6-7 fold increase in activity of *Heterodera schachtii* and *Ditylenchus dipsaci* observed by WALLACE (1958) when the temperature was raised from 50° to 59° F. There was little difference in length of survival between 60° and 70° F., but there was a decrease of 1 month between 70-80° and 80-90°, and a 2 month decrease between 90-100° F. Temperatures of 32° and 40° F. were usually lethal to larvae in 7 and 14 days respectively.

Eggs were more resistant than larvae to temperatures below 50° F., but survived only from 25 to 50% as long as did eggs at 50° F. Survival of eggs as such in any experiment at 60° F. or above was questionable since most of the eggs hatch within the exposure times at these temperatures. At 60° and 70° F. there were no differences in survival between the two stages, but at 80° and 90° F. eggs survived a month longer than the larvae. The direct application to field situations of the specific time limits established for each temperature may be altered by factors nonoperative in laboratory experiments of this type.

Thanks are due to Dr. M. W. Allen of the University of California

for his advice and encouragement during the course of these investigations.

ZUSAMMENFASSUNG

Einfluss der Temperatur auf das Überleben von Arten der Gattung Meloidogyne *in Abwesenheit einer Wirtspflanze*

Der Einfluss von Temperaturen (32 bis 100° F.) auf das Überleben der Eier und Larven von *Meloidogyne incognita acrita* wurde bestimmt. Von den geprüften Temperaturen lag 50° F. dem Optimum für das Überleben von Eiern und Larven am nächsten. Bei dieser Temperatur überlebten beide Stadien länger als 1 Jahr. Das Überleben der Larven bei Temperaturen von 32, 40, 50, 60, 70, 80, 90 und 100° F. betrug 8,7-14, > 365, 120-150, 120-150, 90-120, 45-60 und 21 Tage. Das Überleben der Eier bei denselben Temperaturen (ausgenommen 100° F.) lag bei 90, 90-180, > 365, 120-150, 120-150, 120-150 und 90 Tagen.

M. hapla war im Eistadium resistenter gegenüber einer Temperatur von 32° F. und im Larvenstadium resistenter bei 40° F. als Eier und Larven von *M. incognita acrita* und *M. javanica.*

Versuche, die Larven gegenüber letalen Temperaturen durch eine Reihe subletaler Temperaturen zu beeinflussen, waren erfolglos. Die Schlüpfhöhe der Eier von Wurzelgallenälchen erwies sich als abhängig von der Temperatur. Bei 40 und 50° F. war das Schlüpfen gering und sank innerhalb einiger Tage schnell ab. Bei 60° F. setzte sich das Schlüpfen in fast gleicher Höhe wenigstens 22 Tage fort. Sie erreichte ihren Höhepunkt zwischen dem vierten und dem siebenten Tag bei Temperaturen von 70, 80 und 90° F. und sank dann plötzlich ab. Es bestanden Anzeichen, dass bei 90° F. das Schlüpfen zum Teil behindert wird, aber es setzte sich fort, wenn die Temperaturen erniedrigt wurden.

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BOOK REVIEW

Plant Nematology, edited by J. F. Southey, Technical Bulletin Ministry of Agriculture, Fisheries and Food, London, no. 7, 1959.
Published by Her Majesties Stationery Office, 175 pages, 12 plates, 67 figures. Alphabetical Index, Price 9 s. 6d.

This publication is based on a series of lectures given at a training course in plant nematology for the entomologists of the (British) National Agricultural Advisory Service in 1956. As many as sixteen authors contributed, who, in twenty one well balanced chapters, gave a general introduction to plant nematology and a concise up to date review of the present knowledge about the species which are important in the United Kingdom (and other Western European Countries). There are six sections:

I General Nematology (Anatomy and systematics of plant and soil nematodes)
II The principal genera of plant parasitic nematodes
III The importance of cyst forming eelworms in Agriculture
IV Research problems with Heterodera species
V Control.
VI Laboratory culture and nutrition studies

In accordance with their relative importance cyst forming eelworms are discussed more at length than other species.
The book contains a simplified key to orders and families of plant and soil nematodes, a key to perineal patterns of *Meloidogyne* species, and figures of the nematode species discussed in the text including perineal patterns of root knot nematodes and distinguishing characters of *Heterodera* cysts.

J. W. Seinhorst

SCIENTIFIC NOTES

E. B. Sledge[1]), *The extrusion of saliva from the stylet of the spiral nematode,* Helicotylenchus nannus.

The extrusion of a substance into host tissue from the esophageal glands of nematodes is generally believed to occur. Some experiments indicate this material is enzymatic and functions in extracorporeal digestion (Linford 1937a). These secretions are also thought to cause severe damage to host tissues such as galling of roots by *Meloidogyne* spp. (Linford 1937b).

Linford (1937b) observed the extrusion of saliva from the stylet of *Meloidogyne* sp. females which had been removed from roots and placed in water or a nutrient solution. The saliva formed a definite, but irregular, filament as it passed from the stylet tip into the the surrounding liquid. The entrance of saliva into a host plant cell was not observed in experiments with *Meloidogyne* and a fungal feeder, *Ditylenchus intermedius* (de Mann 1880) Filipjev 1936, but predaceous species of *Aphelenchoides* were observed injecting saliva into their nematode prey (Linford 1937a).

Extrusion of saliva from the stylet of a female *Helicotylenchus nannus* Steiner 1945, into a root hair cell of corn, *Zea Mays* L., has been observed by the writer. Specimens of *H. nannus* were placed on corn seedlings growing in water agar and their feeding habits observed on both roots and root hairs. The nematode oriented itself with the head perpendicular to the longitudinal axis of the root hair and slowly thrust the stylet against the cell wall. Between periods of frequent thrusts of the stylet there were rests of about one minute. Actual penetration of the cell wall by the stylet was not seen because of the curvature of the cell wall and the disposition of the material. During an interval of stylet inactivity a small globule was seen entering the plant cell adjacent to the stylet tip. This globule appeared to be light green under ordinary illumination without filters and more viscous than the hyaline protoplasm of the root hair. It slowly increased in size and became slightly elongated parallel to the long axis of the host cell. Then it moved rapidly away from the stylet tip and broke down into smaller globules which finally became indistinguishable from the cell contents. The median esophageal bulb of the nematode began pulsating some fifteen minutes after the first appearance of the globules of saliva. The pulsations continued for an undetermined period after which the parasite became quiescent and displayed no further feeding activity.

Linford, M. B. (1937a). The feeding of some hollow-stylet nematodes. *Proc. helm. Soc. Wash.* **4**, 41-46.

Linford, M. B. (1937b). The feeding of the root-knot nematode in root tissue and nutrient solutions. *Phytopathology* **27**, 824-835.

F. A. van der Vegte[2]), *A Method for fixing and mounting Nematodes in one Process* .

To save time and to combine the advantages of a temporary water mount and a permanent glycerine mount, use was made of the fixative F.A.A. to which gum arabic and pure glycerine were added. Mounts made in December 1956 are still in excellent condition with all important morphological details (internal organs,

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1) State Plant Board Laboratory, Winter Haven, Florida, U.S.A.

2) Division of Entomology, Section Nematology, Pretoria, South Africa.

lateral fields, stylet) remaining distinct. Mounts can be examined under oil immersion immediately they are made and can be sealed later, making it possible to go from the water-suspension to oil-immersion examination within three minutes. The formulation of the mixture is as follows:

Gum Arabic (Acacia) tears	50 gm.
Distilled water	100 cc.
Formalin 40%	40 cc.
Glycerine (pure)	40 cc.
Alcohol 96%	50 cc.
Glacial Acetic Acid	3

Dissolve the 50 gm. gum arabic tears in 100 cc. distilled water at room temperature, filter the mixture and add the other ingredients. The pH of this mixture is 3.5. It is proposed to name this new mixture ARFAGAL.

Mounting process. Put the living nematode in a drop of the above mixture in a cavity slide and heat until vapour is observed. Place a very small drop of mixture on a clean slide, arrange the nematode in it and cover with a warmed coverslip. Stains can be added to the mixture. At the suggestion of Prof. dr. H. G. Schweickerdt, Chief of Department General Botany of University Pretoria, Orseilline BB, a stain used for plant tissues, was tested and found to be excellent for the staining of nematodes. This substance derived from a certain lichen is so intense that a very minute quantity is required to give satisfactory results. A saturated solution of the powder is made in 3% acetic acid. About 6 drops of this solution in the above mentioned quantity of Arfagal is sufficient.

Neue Anschrift.

Im Laufe des Dezember 1959 wird das Institut für Hackfruchtkrankheiten und Nematodenforschung der Biologischen Bundesanstalt für Land- und Forstwirtschaft ein neues Institutsgebäude beziehen. Die neue Anschrift lautet: Münster/Westf., Toppheideweg 88. Das Institut verfügt dann über genügend Räume, um auch Gäste, die kürzere oder längere Zeit am Institut auf dem Gebiete der Nematodenforschung arbeiten wollen, aufnehmen zu können. Diesbezügliche Anfragen werden rechtzeitig erbeten.

H. GOFFART, B. WEISCHER

Editors' Note.

At their meeting during the Nematology Symposium at Uppsala, the Editors' appointed Dr. J. B. Goodey to the Editorial Board. This last number of Volume 4 has been increased in size by some 36 pages to speed up publication and, in consequence, the first number of Volume 5 may be somewhat smaller. Starting with Volume 5 a new and improved format will be used. Owing to increased costs the printers regret to announce that the subscription price will be raised from *f* 28.— to *f* 32.—.

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